Lewy Body Dementia and the Role of Inflammation

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This dissertation is submitted for the degree of Doctor of Philosophy

Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

It does not exceed the prescribed word limit for the Clinical Medicine and Clinical Veterinary Medicine Degree Committee for a PhD (60,000 words excluding figures, photographs, tables, appendices and bibliography).

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Abstract

Background: Lewy body dementia (LBD), consisting of Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB), is known to make up more than 15% of dementia cases at autopsy, however the clinical prevalence rate is reported to be much lower at around 5-6%. Difficulties with diagnosis and/or lack of specific treatments may contribute to this difference. This study investigated the diagnosis and management pathways of LBD and whether inflammation could play a role in the pathophysiology and hence provide a route for future diagnostic and treatment pathways.

Methods: Clinical diagnostic rates of LBD in clinics across several NHS trusts in East Anglia were reviewed, followed by an in-depth notes review of patients identified with LBD together with age and gender matched controls. A literature review of the current evidence for inflammation in LBD, preceded a case control study to investigate further. Nineteen DLB patients together with 16 age and gender matched healthy controls underwent [11^{C}]PK11195 PET imaging, and the same cohorts, plus an additional 10 matched control subjects underwent peripheral cytokine analysis.

Results: The clinical prevalence rate of LBD was low compared to the known pathology rates, with delays identified in the diagnosis of DLB compared to other dementia subtypes. Delays were also seen between the onset of dementia symptoms and the clinical diagnosis of dementia in Parkinson's disease (PD). The literature review identified studies providing evidence of inflammation in PD but few studies had been carried out in DLB. PET imaging revealed microglial activation negatively correlated with disease severity in DLB, suggesting inflammation occurs early in the disease. DLB patients also showed evidence of differences in cytokine levels compared to healthy controls.

Conclusion: The study showed evidence of inflammatory changes in DLB, providing a potential target for treatment and/or biomarkers, that could assist in increasing clinical diagnostic rates.

Abbreviations

ACE-R	Addenbrooke's Cognitive Exam - Revised
AD	Alzheimer's dementia
ADLs	Activities of daily living
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARSAC	Administration of Radioactive Substances Advisory Committee
BB	UK Brain Bank
BP_{ND}	Non-displaceable binding potential
CAMCOG-R	Cambridge Cognitive Assessment–Revised
Chi Sq	Chi Squared statistical test.
CI	Confidence interval
CNS	Central Nervous System
CPFT	Cambridgeshire and Peterborough Foundation Trust
CRF	Case report form
CSF	Cerebrospinal fluid
CUH	Cambridge University Hospitals Foundation Trust
DaTscan	Dopamine transporter scan
DeNDRoN	Dementias and Neurodegenerative Diseases Research Network
DLB	Dementia with Lewy bodies
DSM	Diagnostic and Statistical Manual of Mental Disorders
FcγR	Fc gamma receptors
FDG	Flurodeoxyglucose
FTD	Frontotemporal dementia
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GWAS	Genome wide association studies
hsCRP	High sensitivity c-reactive protein
ICD	International Classification of Diseases
IFN	Interferon
IL	Interleukin
IP-10	Interferon gamma-induced protein 10
LBD	Lewy body dementia
LPS	Lipopolysaccharide
LRRK2	Leucine-rich repeat kinase 2
MCI	Mild cognitive impairment

MCP	Monocyte chemotactic protein
MCSF	Macrophage colony-stimulating factor
MDC	Macrophage derived chemokine
MDS	Movement Disorder Society
MHCII	Major Histocompatibility Complex class II
MIBG	^{123}Iodine- metaiodobenzylguanidine myocardial scintigraphy
MIP	Macrophage inflammatory protein
MMSE	Mini Mental State Examination
MOCA	Montreal Cognitive Assessment
MPRAGE	Magnetization-prepared rapid acquisition gradient-echo
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NHS	National Health Service
NNUH	Norfolk and Norwich University Hospitals Foundation Trust
OAP	Old Age Psychiatry
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PiB	^{11}C Pittsburgh compound B
PK11195	^{11}C-*R*PK11195
PRR	Pattern-recognition receptor
R&D	Research and Development
RANTES	Regulated on activation, normal T cell expressed and secreted
RBD	REM sleep behaviour disorder
REM	Rapid eye movement
SD	Standard deviation
SPECT	Single-photon emission computed tomography
SUVR	Standardized uptake value ratio
SVM	Support vector machine
TARC	Thymus- and activation-regulated chemokine
TLR	Toll-like receptor
TNFR	Tumour necrosis factor receptor
TNF-α	Tumor necrosis factor α
TREM2	Triggering receptor expressed on myeloid cells 2
TSPO	Translocator protein
UPDRS	Unified Parkinson's Disease Rating Scale
VEGF	Vascular endothelial growth factor
YKL-40	Chitinase-3-like protein 1

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Chapter 1

Lewy Body Dementia Diagnosis - Background

1.1 Introduction

The term Lewy body dementia (LBD) describes two syndromes: dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD). This chapter will describe both conditions and review studies investigating their frequency and prevalence from both clinical and pathological series.

Both DLB and PDD are characterised by Lewy bodies found in the brain on post-mortem in patients who have a clinical dementia syndrome. Lewy bodies were first described by Frederick Lewy in 1912 (Lewy, 1912), who found concentric inclusion bodies characterised by a densely staining core and a pale surrounding halo within the nucleus basalis of Meynert in a patient who had died from Parkinson's disease. Alpha-synuclein has since been discovered as the chief component of Lewy bodies (Spillantini *et al.*, 1997).

Both PDD and DLB share several clinical characteristics, the most common being dementia and a parkinsonian syndrome. Indeed, conventionally they are somewhat arbitrarily distinguished by a one-year rule. Namely that in PDD, the patient must have had idiopathic Parkinson's disease (PD) for at least one year before they are diagnosed with dementia. In DLB, the patient must have either developed dementia before, or within one year of onset of the parkinsonian symptoms. At autopsy the two conditions are difficult, if not impossible, to distinguish and so the distinction has to be made on the clinical history.

Both PDD and DLB have their own internationally agreed consensus clinical diagnostic criteria. DLB is diagnosed using the 2005 consensus criteria (McKeith *et al.*, 2005). All subjects must first satisfy the criteria for dementia, defined as a progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational function.

Table 1.1 DLB Diagnostic Criteria (McKeith *et al.*, 2005)

Core Criteria	Suggestive Features
Recurrent visual hallucinations	Rapid eye movement (REM) sleep behaviour disorder
Fluctuating cognition	Abnormal dopamine transporter scan (DaTscan)
Spontaneous features of parkinsonism	Severe neuroleptic sensitivity

A 'probable' DLB diagnosis is made when either two core criteria or one core and one suggestive criteria are present (see Table 1.1 for the list of core criteria and suggestive features). A lower threshold 'possible' DLB diagnosis only requires the presence of one of the six core or suggestive features listed. Newer criteria for the diagnosis of DLB were published in June 2017 (McKeith *et al.*, 2017), following the completion of the studies in this thesis and they are discussed further in Chapter 4.

The PDD criteria were published by a Movement Disorder Society (MDS) task force in 2007 (Emre *et al.*, 2007) before a further clarification was released to aid in its diagnosis by the same group (Dubois *et al.*, 2007). They list criteria that define dementia on the background of PD. Multi-domain cognitive impairments are required which are not explained by other plausible causes such as major depression or delirium.

DLB has recently been incorporated into the International Classification of Diseases (ICD) 9 and 10 (WHO, 2016) however ICD-9 does not specifically include PDD as a dementia subtype, whereas ICD-10 includes PDD as a subtype of DLB. The Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 (American Psychiatric Association, 2013) has also recently incorporated DLB.

Autopsy studies suggest that LBD is the second commonest form of dementia after AD, responsible for 15-20% of cases (Perry *et al.*, 1989; Oinas *et al.*, 2007). However, clinical studies consistently show a low rate of prevalence, at the 5-10% level (Vann Jones and O'Brien, 2014; D. B. Hogan *et al.*, 2016; Yue *et al.*, 2016), suggesting difficulty in diagnosing these conditions. This chapter looks at this discrepancy in depth.

1.2 Early Pathological Studies

Early autopsy studies of patients with dementia identified a group of subjects that shared the amyloid pathology of Alzheimer's dementia (AD) and the subcortical neuron loss of PD but did not exhibit significant tau pathology, nor the severity in neuron loss in the substantia nigra seen in these two conditions respectively. This group was classified as having a senile dementia of Lewy body type, forming 15-20% of demented subjects that went to autopsy (Perry *et al.*, 1989, 1990). It should be noted that these studies did not include cases of 'diffuse' Lewy body disease in this figure, as this was considered a separate entity, with higher amounts of neocortical Lewy bodies. However, diffuse Lewy body disease has since been added to the spectrum of disorders considered to be DLB (McKeith *et al.*, 1996). Adding the diffuse DLB group in these early studies, would have increased the proportion of dementia cases with DLB.

Another issue in these early papers is the attempt to separate the diagnosis of DLB and PDD pathologically based on the severity of substantia nigra pathology. Parkinsonism is a core diagnostic criteria of DLB and the involvement of the substantia nigra in DLB is not disputed, although the severity of involvement does vary (McKeith *et al.*, 2005). The authors admitted that including all the patients with Lewy body disorders and associated dementia: PDD and diffuse Lewy body disease, as well as PD with AD (the latter defined as AD pathology but with severe neuron loss and Lewy bodies in the substantia nigra and other subcortical nuclei only) would lead to a number that is even higher at 25% (Perry *et al.*, 1990). In contrast, the inclusion of patients who have co-existing moderate to severe amyloid pathology, and in one case severe neurofibrillary tangles, could have inflated the numbers considered to have DLB in these studies. However, at the time there were no clear pathological (or even clinical) diagnostic criteria for DLB and there would have been difficulty in determining which patients with both DLB and AD pathologies had which disorder.

Hence early autopsy studies suggested LBD represented about 25% of dementia cases but did not address the relevance of co-existing AD pathology that could have inflated this figure.

1.3 New Pathological Criteria

In 1996 the report of the DLB Consortium suggested the first set of both clinical and pathological criteria for DLB. Pathologically, the presence of Lewy bodies anywhere in the brain of a patient diagnosed clinically with dementia (McKeith *et al.*, 1996) was sufficient for a diagnosis of DLB. Whilst these simplistic criteria allow for the inclusion of a wide range of cases, they also include patients who clinically have an AD like profile with no DLB symptoms. Three different pathological types were introduced:

1. 'brainstem', where Lewy bodies are mainly found in the brainstem with no cortical involvement;
2. 'limbic', where there are Lewy bodies mainly in the amygdala and cingulate, with no parietal lobe involvement; and
3. 'diffuse cortical', where Lewy bodies are found throughout the cortex as well as the limbic and brainstem areas.

In 2005, the DLB Consortium reassembled and tried to address the question of how co-existing AD pathology affected the likelihood of a patient with dementia clinically having DLB (McKeith *et al.*, 2005). Up to 60% of AD cases were reported to be considered to meet the pathological criteria for DLB, however as the vast majority of these patients did not have the DLB clinical syndrome, this led to the clinical criteria being deemed inadequate in terms of sensitivity (McKeith *et al.*, 2005). Hence the consortium took steps to take into account AD pathology when determining the extent to which Lewy body pathology explained the clinical DLB syndrome. They introduced new pathological criteria that suggested such likelihood was "directly related to the severity of Lewy related pathology, and inversely related to the severity of concurrent Alzheimer's disease type pathology". The three pathological classifications for DLB were kept: brainstem, limbic and diffuse cortical but the likelihood of a patient with dementia having DLB was attributed to being "low", "intermediate" or "high" dependent on the severity of Lewy body and AD pathology in these different categories.

A patient with brainstem Lewy bodies was said to have a low likelihood of clinically having DLB if they had any element of AD pathology. Whereas, patients with diffuse cortical Lewy bodies could have even high levels of AD pathology and still have an intermediate probability of having DLB. Patients with the limbic type sat in the middle in terms of the likelihood of a DLB syndrome if there was co-existing AD pathology (McKeith *et al.*, 2005).

Alpha-synuclein immunohistochemistry was also recommended instead of haematoxylin and eosin staining or ubiquitin staining for Lewy body quantification in order to increase detection of Lewy bodies (McKeith *et al.*, 2005).

1.4 Recent Pathological Studies

The new pathology criteria do not appear to have diminished the proportion of dementia cases with a pathological diagnosis of DLB reported at autopsy. The earliest study to assess the utility of the new pathological criteria was carried out by Fujimi and colleagues (Fujimi *et al.*, 2008). It reported the autopsy results of 205 consecutive patients with dementia. 32 (15.6%) were found to have an intermediate and high likelihood of DLB and the authors viewed this group of patients to have a pathological diagnosis of DLB. A further 27 (13.2%) had Lewy body pathology but were thought to have a low likelihood of DLB. The authors however found the three pathological classifications (brainstem, limbic and diffuse cortical) inadequate as about half of their 59 cases did not fit well into the criteria. Lewy body distribution was found to be highly variable and not as easily amenable to classification as AD pathology. PDD patients, of which there were 8, were excluded from detailed assessment.

Another difficulty was with patients exhibiting AD pathology but with a co-existing heavy burden of Lewy bodies in the amygdala and thus in the limbic category, yet often without any core features of DLB clinically. The authors proposed that this group required further investigation with respect to the clinical significance of Lewy bodies found in the amygdala. A previous study investigating co-existing Lewy body pathology in Alzheimer's disease patients, had found that 62 (or 18%) of 347 consecutive AD cases had co-existing Lewy bodies within the amygdala with minimal Lewy body deposits elsewhere in the brain. A comparison with AD patients without co-existing Lewy bodies found no significant differences in their clinical features (Uchikado *et al.*, 2006). A further category of "amygdala predominant" has since been suggested for addition to the pathological subtypes, after another group showed this subtype accounted for 20% of dementia cases with Lewy body pathology at autopsy but didn't fit into any of the 2005 pathological categories (Leverenz *et al.*, 2008).

Another interesting aspect of the Fujimi study (Fujimi *et al.*, 2008) was that the group with diffuse cortical Lewy bodies and also severe AD pathology had the highest frequency of core clinical features of DLB but were categorised as having an intermediate

likelihood of DLB pathologically. This would suggest that those with severe dual AD and DLB pathologies are likely clinically to be diagnosed with DLB, but that a mixed AD and DLB diagnosis may be more appropriate in some of these cases. AD pathology was seen to increase with age as expected, as did DLB pathology, but the greater prevalence of AD with age meant the likelihood of DLB reduced with age.

Another interesting aspect of the study was the equal spread of Lewy body prevalence across the genders. However, the distribution of the pathology varied - with females having more diffuse cortical Lewy bodies compared to males and females also having a higher prevalence of AD pathology. Hence any gender differences seen clinically could be due to increased AD pathology in females particularly with age, leading to predominantly AD symptoms. Again further research needs to be carried out as this study involved only 59 DLB subjects (Fujimi *et al.*, 2008).

Another four autopsy studies have been reported since the release of the 2005 pathological criteria for DLB, but only one considered the clinical history to determine if some of the cases were PDD rather than DLB. This Austrian hospital based autopsy series (Jellinger and Attems, 2011) retrospectively assessed 1,100 dementia cases aged 70 or over who died between 1990 and 2007. The study showed the percentage of DLB cases as a proportion of all dementia cases, to be 8.5% (where AD pathology was only found at low levels and quantified as Braak stage 4 or less) with another 8.9% having concomitant DLB and AD pathology (with Braak stage of 5 or 6). Interestingly for both groups the percentage of cases diagnosed clinically as having DLB pathology was lower, at 10%. However given many of the cases would have died before the DLB consensus criteria were established, this is likely to represent a lack of recognition of the condition at the time of diagnosis. Indeed, the majority of both these groups were clinically diagnosed with PDD, a condition which was much better recognised at the time. However, this study was limited by a lack of clinical information for the autopsied patients. Interestingly the percentage of cases with DLB pathology again fell with age.

The other autopsy studies did not differentiate DLB from PDD using clinical records, so any reference to DLB has to be considered to be a reference to LBD as a whole. Oinas and colleagues (Oinas *et al.*, 2007) reclassified autopsy cases with the newer 2005 criteria and newer staining techniques and found a prevalence rate of 20% (11 of 55) of pathological DLB in consecutive hospitalised dementia cases, but all of these cases had co-existing AD pathology and nine had high levels of AD pathology. One of the strengths

of this study was the broad range of ages of the patients' autopsied, with half younger than 65.

A study comparing consecutive autopsy cases in community and clinic cohorts found differences in Lewy body pathology between the two cohorts, though the actual pathological prevalence rates of DLB were not stated (Schneider *et al.*, 2009). Those seen in the specialty memory clinic were reported to have increased Lewy body pathology, suggesting a referral bias – the community and clinic cohorts both had a similar autopsy rate and autopsies were all carried out at the same centre, reducing other potential sources of bias such as operator bias or selection bias. However, the study did not take into account the 2005 pathological criteria for assessing likelihood of DLB nor did it mention the diagnosis of PDD. However, it did show that the prevalence of patients with neocortical Lewy bodies, which significantly increase the likelihood of a DLB diagnosis irrespective of the presence of AD pathology, formed about the same proportion of dementia patients in each cohort - at 20%.

The "90+study" by Corrada and colleagues (Corrada, Berlau and Kawas, 2012) found that 13 out of 64 (21%) patients aged 90 and over from a retirement community who underwent autopsy had diffuse Lewy body disease. However, only 1.5% of patients with dementia were diagnosed with Lewy body disease clinically prior to autopsy. The study did not appear to assess for the other categories of DLB (limbic and brainstem) nor state what histological methods were used for assessing Lewy bodies. It was not stated that the diagnosis of PDD was considered. Given the age group and lack of detail around the methodology, it is difficult to extrapolate the findings to the general dementia population.

To summarise, the two studies (Fujimi *et al.*, 2008; Jellinger and Attems, 2011) using the 2005 pathological criteria and clinical history to identify pathological DLB in autopsy studies, suggest the prevalence of dementia subjects with intermediate or high likelihood of DLB to be about 15-20%. The only other study which used the 2005 criteria reported a DLB prevalence of 20%, though it did not verify the clinical history using medical records. Two further studies which chose not to use such criteria, still reported the presence of diffuse cortical Lewy bodies in their dementia cohorts, which by definition meant at least an intermediate likelihood of DLB, to be similarly about 20%.

This 15-20% prevalence figure for DLB however is tempered by major methodology problems, including: a lack of recognition of PDD as a diagnostic entity (in 3 out of the 5 studies), small sample sizes and differing or unstated histological methods. In addition, a large number of autopsy studies of patients with dementia carried out recently but not

discussed here do not even take into account the DLB consensus criteria for its pathological diagnosis and therefore did not report on its prevalence specifically, for example studies by Brunnström and colleagues and Leiros and colleagues (Brunnström *et al.*, 2009; Leiros *et al.*, 2016).

Another difficulty with interpreting autopsy studies where consecutive cases are used to measure prevalence rates is the likely selection bias inherent in patients referred for autopsy. Complex cases where diagnoses are doubted are much more likely to be referred, in addition some centres have a specialist interest in Lewy body disease and will have a higher suspicion rate for the diagnosis. DLB is therefore likely to be more common in these sample populations.

Despite these difficulties, the evidence that we do have from pathological studies suggests that DLB forms about 15-20% of dementia cases as a whole. As most studies have not distinguished DLB from PDD at autopsy, we would need to take this figure to represent DLB and PDD combined (LBD) rather than DLB specifically. Albeit the studies that do take the distinction into account, still state a DLB prevalence of 15-20%. Hence LBD as a whole appears to represent a significant proportion of dementia. However clinically the proportion appears to be much lower and this is discussed below.

1.5 DLB Clinical Prevalence

There have been two reviews of the literature carried out recently to attempt to identify the rate of DLB diagnosed clinically (without pathological verification). The two studies used different methodologies and therefore represent a robust survey of the current prevalence of DLB in the community.

In the first study (Vann Jones and O'Brien, 2014), a literature search of PubMed carried out in 2013, revealed 18 population prevalence studies and 10 clinical prevalence studies. Papers were only included if they stated that the DLB consensus criteria from either 1996 or 2005 were used in their methods. The mean proportion of DLB cases within secondary care patients with dementia, was found to be higher at 7.5% than the mean rate in population studies, which was 4.2%. This may be because of the higher burden of symptoms in DLB that means a higher proportion attend secondary care. However, it could also represent the difficulty in differentiating AD and DLB in the community, with specialists more likely to pick up the extra-pyramidal signs in DLB and also the more difficult to identify clinical symptoms of fluctuation and REM sleep

behaviour disorder. There was also an increased prevalence found in those studies using the 2005 criteria compared to the 1996 criteria, of 8.2% compared to 3.7%. The addition of a DaTscan and REM behaviour disorder to the criteria could have played a part in increasing diagnostic rates using the 2005 criteria.

It was also noted that in three studies where the primary aim was to identify the prevalence of DLB, the prevalence rates within dementia patients was much higher: 19.9%-24.9%. Suggesting that if specific evidence of DLB was sought, this resulted in a higher diagnostic rate (Vann Jones and O'Brien, 2014). Overall the results of this literature review suggest there is difficulty in diagnosing DLB, particularly outside of secondary care, but also where there is a lack of awareness or expertise in the core features of the disease.

In the second review by Hogan and colleagues (Hogan et al. 2016), a literature search was carried out on MEDLINE and EMBASE, revealed 17 prevalence studies, of which 10 were in the earlier study by Vann Jones. As a proportion of all dementia cases, DLB made up a wide range of prevalence of 0.3 to 24.4%, with no difference found in this review between those studies using the 1996 criteria compared to the 2005 criteria. A pooled prevalence figure was not attempted due to the varying methodologies used in the underlying papers. It was noted in this review that the majority of the studies did not report on whether it was "possible" or "probable" DLB prevalence being reported. Another difficulty was the inconsistency in the classification of mixed AD and DLB disease, where this was considered at all. The authors concluded that clinically defined DLB accounted for "about 5% of all dementia cases encountered in older populations".

1.6 Recent DLB Clinical Prevalence Studies

An updated literature search was carried out to identify if further studies on the prevalence of DLB as a proportion of dementia cases has been carried out since the review by Hogan and colleagues. The search also included papers on the prevalence of PDD.

A search of PubMed was carried out on 17 February 2017, looking for the following terms in the Title or Abstract:

1. "LEWY" OR "PARKINSON*", AND
2. "INCID* OR "PREVAL*", AND

3. "DEMENTIA"

Only articles written in English were included. Table 1.2 shows a summary of the articles on DLB prevalence that have been published since these two reviews.

Yue and colleagues (Yue *et al.*, 2016) found the prevalence of DLB in rural China to be 10.1% using an initial door to door survey to identify dementia, followed by a clinical review to confirm the subtype. The authors carried out the survey with the aim of identifying the proportion of DLB cases in dementia as a whole. Another population study by Ikejima and colleagues (Ikejima *et al.*, 2012) found a DLB prevalence of 4.6% in over 65s with dementia in rural Japan. Once again an initial screening procedure was followed by a clinical assessment, but this study was not focussed on identifying DLB cases.

Another study by Bonanni and colleagues (Bonanni *et al.*, 2017) did not state the prevalence of DLB in percentage terms but found frontotemporal dementia (FTD) to be more common than DLB and AD. As FTD is an uncommon disorder mainly affecting people below the age of 65, with a prevalence of about 2.5% in the over 65s (Hogan *et al.*, 2016), this was a surprising result. The study used email questionnaires and had limited success with a low 25% response rate. The questionnaire also focussed on the opinions of clinicians about the diagnosis of DLB, AD and FTD specifically, which may have biased the results.

Two further studies did not distinguish between DLB and PDD and found the rate of LBD in dementia patients to be 5.4% and 0.8% (Perera *et al.*, 2016; Goodman *et al.*, 2017). They are discussed below under PDD prevalence.

To summarise, the two systematic reviews suggest DLB has a prevalence of 4.2% to 5% of all dementia cases, which would mean that of the 800,000 dementia patients in the UK (Prince *et al.*, 2014), 33,000-40,000 would have DLB. Three further studies have additionally investigated the presence of DLB as a proportion of dementia, but only two (Ikejima *et al.*, 2012; Yue *et al.*, 2016) had robust methodology involving clinical assessments. One study sought to identify DLB specifically and found a slightly higher rate of 10%. The other study did not and found a similar rate to the earlier reviews of 4.6%.

A DLB prevalence rate of about 5% would be substantially lower than the 15-20% rate found in autopsy studies. However, as this latter autopsy rate is for LBD, we would also need to look at the clinical prevalence of PDD as a proportion of dementia cases as they would also contribute to the number reported.

1.7 PDD Clinical Prevalence

Aarsland and colleagues carried out a systematic literature review of both the prevalence of dementia in Parkinson's disease and also the prevalence of PDD as a percentage of dementia cases as a whole (Aarsland, Zaccai and Brayne, 2005). From the 12 studies on the former that satisfied their inclusion criteria, a mean proportion of 24.5% had a diagnosis of dementia in Parkinson's disease. A further 24 studies looked at PDD as a proportion of dementia, with a mean proportion reported of 3.6%.

This was a thorough review using wide search criteria as well as of references in the articles found, with no restrictions on language or time frame. The authors noted that several studies included in the review did not differentiate DLB from PDD, or the timing of the onset of parkinsonian symptoms and cognitive impairment, leading to the possibility that some patients reported as PDD should have been diagnosed with DLB or vice versa, that some patients with DLB should have been diagnosed as PDD. It should also be noted that the current MDS criteria for PDD (Dubois *et al.*, 2007; Emre *et al.*, 2007) had not been established at the time of the review.

Interestingly, in the studies reviewed by Aarsland and colleagues the rate of dementia did not correlate significantly with the mean age of PD subjects. It was noted however that these studies rarely stratified dementia incidence in PD according to age (Aarsland, Zaccai and Brayne, 2005). In addition, it is well established that dementia risk increases with age, an association that has previously been reported in PD specifically (Levy *et al.*, 2002).

1.8 Recent PDD Clinical Prevalence Studies

Since the publication of this review, the literature search described above discovered a further eleven studies that assessed the rate of dementia in PD (see Table 1.3) at a certain point in time. Nine out of the eleven reported a prevalence of dementia in PD patients of 20-30% (the two outliers were one study which reported a rate of 12% and another of 38%). This range is consistent with the 24.5% reported by Aarsland in his earlier review.

Only one study used the established MDS criteria for dementia in PD (Dubois *et al.*, 2007; Emre *et al.*, 2007). This clinic based study (Wang *et al.*, 2014) of 901 PD patients found a dementia prevalence of 21%, again largely consistent with the figure of 24.5%.

The range of 20-30% was consistent despite the diverse methods used in these studies. Variations occurred in searching for PD cases (including clinic based and population based studies as well as an insurance record survey) as well as the means by which dementia was assessed. The latter could be a simple cognitive assessment with no regard to functional ability but also a more comprehensive full neurological exam, cognitive battery and brain imaging carried out by clinical specialists. Additionally, there were demographic differences in the sample population in terms of age groups and also geographical regions in the world and subsequently their social environment and education status.

This would suggest that the consistent estimate of 20-30% of PD patients having dementia at any given time, is likely to be very close to the true prevalence. This would not conflict with longitudinal studies that look at the number of patients with PD who are eventually affected by dementia. Of 130 PD patients that were followed up for 20 years, 75% developed dementia. In total only 30 survived the entire period of 20 years and 25 (83%) of these were diagnosed with dementia (Hely *et al.*, 2008).

There were no further studies more recent than the review that looked specifically at Parkinson's disease dementia as a proportion of all dementia cases. Three studies stated the combined LBD prevalence as a proportion of dementia, not making a distinction between DLB and PDD, these are also listed in Table 1.3 and further discussed below.

Goodman and colleagues' (Goodman *et al.*, 2017) survey of Medicare claims in the US, found a combined LBD rate of 5.4% within dementia cases as a whole. However, diagnoses were obtained from insurance information where diagnoses were coded according to ICD-9, which lists dementia with Lewy bodies as a dementia subtype but does not have a code for dementia in PD. The study team did not independently verify each diagnoses. In addition, a number of cases were entered in more than one diagnostic category. The study team also used the terms LBD and DLB interchangeably and therefore it is not clear whether the authors intended to include PDD within their figures. Nevertheless, over 3 million cases of dementia were included, making this an unusually large data set.

A study of dementia in rural Japan found an LBD prevalence rate in the under 65s of 6.2% using a postal survey (Ikejima *et al.*, 2009). Both DLB and PDD were stated to be diagnosed using the "revised criteria for the clinical diagnosis of dementia with Lewy bodies". Which would suggest that the authors have not looked for PDD within the study,

but have instead assumed that both diseases are the same and are diagnosed using the same criteria (this study is also summarised in Table 1.2).

A study of clinical records and death certificates in a large health service in London reported LBD as making up 0.8% of patients with dementia (Perera *et al.*, 2016). However, there were also a large proportion who had unspecified dementia (25.6%) and the results did not include clinical verification of the diagnosis by the study team. PDD was not specifically mentioned and it is likely that most of these patients had DLB. Indeed, Parkinson's disease was not mentioned in the study report at all with the authors omitting to look for PD in the health records or on death certificates.

Despite their methodological issues, the newer studies suggest the combined DLB and PDD rates of 5.4%, 6.2% and 0.8%. With the latter figure likely to be low due to the large number of cases that did not have a dementia subtype in their findings. Excluding this, the other results would not conflict with the figure for PDD as a proportion of all dementia cases of 3.6% found in Aarsland's review published in 2005.

1.9 Lewy Body Dementia Pathological Rates Are Higher Than Clinically Diagnosed Rates

If the 3.6% found in Aarsland's review is a reasonable estimate of clinically diagnosed PDD as a proportion of dementia cases and similarly the 5% from the review by Hogan and colleagues represents a reasonable estimate of clinically diagnosed DLB cases, the combined LBD clinical prevalence rate would be 8.6%. A figure that is significantly lower than the reported 15-20% of dementia cases ascribed to LBD at autopsy.

For DLB, the reasons for a large discrepancy between clinically diagnosed and pathologically confirmed cases are likely to include the following:

a) the lack of awareness by clinicians of how common DLB is and/or the symptoms of DLB,
b) the lack of coding of the DLB diagnosis under major classification systems such as ICD or DSM, until very recently (2012 and 2013 respectively),
c) the difficulties in diagnosing the condition clinically: features such as REM sleep behaviour disorder and cognitive fluctuation require careful clinical assessment (with currently no validated or widely used scales), subtle extra-pyramidal signs

may be missed, and the assessment of visual hallucinations has to be by the patient's (or carer's) history alone,

d) that although biomarkers for DLB exist, for example: an abnormal DaTscan has been shown to have a higher than 80% specificity and sensitivity in differentiating LBD from other dementia subtypes (Brigo, Turri and Tinazzi, 2015), and the myocardial scintigraphy SPECT tracer 123I-MIBGiodine-123-meta-iodobenzylguanidine has also been shown to have high specificity for DLB (Watson and Colloby, 2016), these are not widely available or commonly used in many countries, again making diagnosis harder,
e) the complex nature of the relationship between AD pathology and Lewy body pathology and the resultant clinical syndrome – some DLB patients may not prominently display the classic DLB features and so appear to have AD,
f) the perceived lack of specific treatment options for DLB reducing the motivation for clinicians to look for the DLB diagnosis, when the treatment will just be the same as for AD, and
g) the higher proportion of DLB cases (compared to other dementia subtypes) which may be referred for autopsy, due to the complex nature of their symptoms, and possibly a higher mortality, leading to an over-representation in the autopsy figures.

A study of the current rates of diagnosis of DLB in clinical practice, together with the diagnostic and management pathways associated with the diagnosis compared to other non-LBD subtypes, would show how clinicians currently approach the condition in clinical practice and therefore reveal which of the possible reasons discussed above (or any other reason) are the main drivers behind this mismatch.

Similarly, with respect to PDD, from the clinical studies on the prevalence of dementia in idiopathic Parkinson's disease, it would be expected that between 20-30% of PD patients would have PDD. There are 127,000 reported patients with PD in the UK (Parkinson's UK, 2009). Hence about 25,000 – 38,000 people in the UK should have PDD. In terms of the proportion of all dementia cases that have PDD, if there are 800,000 dementia patients in the UK (Prince *et al.*, 2014) and 25,000-38,000 have PDD, this would be 3.1-4.8% in percentage terms). This would be very consistent with the PDD prevalence rate (as a proportion of all dementia cases) found by Aarsland and colleagues of 3.6%.

Alternatively, if the autopsy rates are correct and 15-20% of dementia cases have LBD, it is possible that there are many more people with PDD as the underlying cause of their

dementia than the higher estimate of 4.8% (i.e. even if 30% of all PD patients are diagnosed with dementia). However, as previously stated, despite the large differences in methodology, 20-30% was consistently found as the proportion of PD cases with dementia, suggesting that this is close to the true prevalence rate of dementia in PD.

Nevertheless, it would be important to know if in clinical practice 20-30% of PD patients are indeed being diagnosed with dementia. If so, this would suggest that the discrepancy may have a different cause. It could be for example that cases of idiopathic Parkinson's disease dementia may be being diagnosed inaccurately as other forms of dementia - for example: vascular parkinsonism combined with vascular dementia.

1.10 Conclusion

In summary, the clinical literature suggest that DLB forms 5% of all dementia cases and PDD 3.6%, meaning LBD as a whole forms 8.6% of all dementia. At autopsy, the proportion is 15-20%, hence half or more of cases do not appear to be diagnosed clinically.

The next chapter explores this further and describes a study of the clinical diagnostic rates of DLB and dementia in Parkinson's disease in the East Anglia region of the UK. A detailed further analysis of the diagnostic pathway and management in individual cases, compared to non-DLB and non-dementia PD cases respectively, will be carried out to understand what may be the key drivers behind the mismatch between pathological rates of LBD and clinically diagnosed rates.

Table 1.2 Recent studies of prevalence of DLB as a proportion of all dementia (study marked # was included in the reviews by Hogan et al. however it is included here as it quoted a figure for LBD rather than DLB alone).

Reference	Sample Population Description	Sample Size	Diagnostic Method	DLB Rate as Proportion of All Dementia	Limitations
(Bonanni *et al.*, 2017) PMID: 27624723	Dementia cases from 135 national dementia centres in Italy	Not stated specifically, but at least 50,000 dementia cases	Emailed questionnaire to clinicians	DLB prevalence not stated in percentage terms	1. FTD found to be more common than DLB (no prior study has reported this finding) 2. Prevalence rate is not reported, only a quarter of dementia centres reported back
(Yue *et al.*, 2016) PMID: 27264344	Over 60s in one county in Rural China	5542 members of general population	Door to door initially, then clinical exam, MRI (or CT if contra-indicated), and PET imaging (amyloid and FDG) where deemed necessary; used 2005	10.1% (58 of 574 dementia cases) were DLB	1. Study team were actively looking for DLB in the population

			consensus criteria for DLB		
(Goodman *et al.*, 2017) PMID: 27172148	Persons over 65 who made claims under Medicare as reported by Centres for Medicare & Medicaid	21.6 million of US population, leading to 3.1 million people with dementia	Identified claims data linked to specific dementia codes	5.4% of dementia cases had LBD	1. Dementia diagnoses were not mutually exclusive, with some cases placed in multiple categories, so true figure likely to be lower 2. Did not distinguish PDD/DLB
(Perera *et al.*, 2016) PMID: 27146301	Patients aged over 65, with a clinical diagnosis of dementia within South London and Maudsley NHS Foundation Trust, over the course of 13 years, who had died	7115 deceased patients	Diagnosis on death certificate and in clinical records	0.8% of clinically diagnosed dementia cases were LBD	1. Small sample 2. Did not distinguish PDD/DLB
(Ikejima *et al.*, 2012) PMID: 22712646	General population in seven rural areas in Japan	768 dementia cases from 3394 participants (from a population of 108, 721 over 65s in total)	Initial screening process, followed by clinical examination by psychiatrist together with MRI; used revised 2005 DLB criteria	4.6% of dementia cases had DLB	1. One of the seven regions reported 95% AD with the remaining 5% classified as ‘mixed’, raising doubts about accuracy of overall figure; no discussion about

					this outlier region was made in the paper
# (Ikejima *et al.*, 2009) PMID: 19478230	285 institutions in one prefecture in Japan with a population of 2.97 million	717 patients with early onset dementia (i.e. onset under 65)	Postal questionnaire, followed by further medical data request, with further 50% having medical note review; DLB diagnosed according to 2005 criteria; PDD criteria stated as the same as DLB criteria	6.2% had DLB or PDD	1. Only covers young onset dementia 2. No PDD criteria listed 3. Postal survey without any clinical assessment of dementia patients

Table 1.3 Recent studies of dementia prevalence in Idiopathic Parkinson's disease

Reference	Sample Population Description	Number of PD cases at Baseline (and diagnostic method)	Method for diagnosing PDD	Dementia Prevalence Rate	Limitations
(Athey, Porter and Walker, 2005) PMID: 15863411	Area of North Tyneside with a population of 108,597	135 cases of PD (diagnostic method not stated)	MMSE and CAMCOG-R (Cambridge Cognitive Assessment–Revised)	23% to 34% (31 to 46/135)	1. Dementia diagnosis based on cognitive instruments alone not functional ability 2. Did not use the MDS PDD criteria (not yet available) 3. Diagnostic criteria for PD not stated
(De Lau *et al.*, 2005) PMID: 16087767	6969 subjects aged 55 years and older in general population	312 dementia cases (no subtypes stated, 67 cases of PD without dementia at baseline (all participants screened for parkinsonism and if positive with UPDRS, followed by	All had MMSE and Geriatric Mental State Schedule, which if positive, followed by Cambridge Examination of Mental Disorders in the Elderly; if required, neuropsychology and if possible MRI;	22% of PD cases at baseline in longitudinal study had dementia	1. Number of total PDD cases at baseline not clear from results and methodology – numbers are inconsistent; the percentage was however stated as 22% 2. Did not use MDS criteria for PDD (not yet available) or BB criteria to assess for PD

		clinical examination by neurologist)	diagnosis made using DSM-III criteria.		3. Not clear how population assessed for PD from the general population were chosen
(Riedel *et al.*, 2008) PMID: 18204803	1749 clinic based patients in Germany with Parkinsonian Syndromes from 315 neurology outpatient settings	873 PD patients (diagnosed using UK Brain Bank (BB) criteria)	DSM IV criteria for dementia used in combination with MMSE and clock drawing test	29% of PD cases had dementia	1. Did not use MDS PDD criteria (not yet available) 2. Clinic based study
(Buter *et al.*, 2008) PMID: 18362281	220,000 inhabitants in Rogaland County, Western Norway	233 PD cases at baseline (diagnosis of PD made if 2/4 of resting tremor, rigidity, akinesia, postural instability present, plus a moderate response to dopamine agent and no other cause of parkinsonism noted)	Interview with patient and informant with DSM-III-R criteria used plus administration of three cognitive rating scales, including MMSE	27% (62/233) were diagnosed with dementia at baseline in longitudinal study	1. Did not use MDS PDD criteria (not yet available). 2. Dementia diagnosis based on cognitive instruments alone not functional ability. 3. PD not diagnosed using BB criteria

(Hu *et al.*, 2011) PMID: 21394490	248,606 registered patients at GP Practices in Milton Keynes, UK	202 patients with PD (diagnosed using the BB criteria by neurologists)	PD dementia defined as an ACE-R score of <74 in patients over the age of 65 years, and score of <82 in patients under the age of 65 years	25% (44/174) of PD patients able to do cognitive tests rated as having dementia	1. Non-standard dementia cut-offs 2. ADLS/ functional ability not considered when making the dementia diagnosis 3. Did not use MDS PDD criteria 4. 28 patients unable to do cognitive tests with reasons not given, meaning dementia prevalence could have been anywhere between 22 & 36%
(Khedr *et al.*, 2013) PMID: 22902078	112 Patients with PD attending out-patients' clinic of Assiut University Hospital, Assiut, Egypt	112 PD patients (diagnosed using BB criteria)	MMSE was used to assess for dementia, but no further details given.	22% of PD patients described as having dementia	1. Not clear who assessed patients for PD or dementia 2. Not clear how dementia was diagnosed 3. Small clinic based population
(Sanyal, Banerjee and Rao, 2014)	Outpatients of the neuromedicine clinic of National	250 patients with PD at baseline (diagnosed by	Interview of patient and carer by neurologist; battery of cognitive	27% (68/250) of PD patients at	1. Did not use MDS PDD criteria

PMID: 24771763	Neurosciences Centre, Kolkata	neurologists using BB criteria)	assessments including MMSE applied, with DSM-III-R criteria used for diagnosis of dementia, plus MMSE score lower than the corresponding quartile based on age and education and a score of 2 or higher on the UPDRS intellectual impairment item	baseline in longitudinal study had dementia.	2. Hospital based clinic study, therefore a narrow sample group 3. Screening procedure for initial assessment not detailed in methods
(Wang *et al.*, 2014) PMID: 24550669	901 patients from 42 university-affiliated hospitals throughout seven cities in China	901 patients with PD (satisfying BB criteria as assessed by local clinicians and confirmed by Principal Investigator)	Assessed for dementia using MMSE, MOCA and further battery of cognitive tests; PDD diagnosed using MDS criteria for PDD; diagnoses confirmed by Principal Investigator	21% (193/901) of PD patients diagnosed with PDD	1. Clinic based study

(Oh *et al.*, 2016) PMID: 25656841	1200 patients with PD from 12 hospitals in Korea	1200 patients with PD (diagnosed using BB criteria)	Dementia diagnosed using DSM-IV criteria; Mini-Mental State Examination and Clinical Dementia Rating scale used to assess cognition	38% (460/1200) of patients rated to have dementia	1. Not clear who carried out assessments for PD or dementia 2. Hospital based clinic study 3. Did not use MDS PDD criteria
(Cereda *et al.*, 2016) PMID: 26952697	6599 patients known to the Parkinson Institute-Milan patient database, constituting PD patients from the Lombardy region of Italy	6599 patients (diagnosed using BB criteria evaluated and followed up by same neurologist)	Dementia diagnosed using DSM-IV criteria	12% of PD patients diagnosed with dementia	1. Did not use MDS criteria for PDD 2. Retrospective study 3. Hospital based clinic study 4. Methods not fully clear about how patients were rated as having dementia for study purposes
(Riedel *et al.*, 2016) PMID: 26764603	815,573 insurance holders aged 65 years or over on German Pharmaco-epidemiological Research Database	10,596 PD cases determined by coding at one inpatient or outpatient setting with outpatients confirmed by a subsequent diagnosis of PD or by	Dementia determined by coding of dementia within 365 days of the date of determination of PD	28% of PD cases coded as having dementia	1. No standardised assessment battery by study clinicians, the rates are based solely on insurance data coding 2. No clinical verification and therefore some cases could

		a prescription of an antiparkinsonian drug within 12 months			have been coded as PD, then dementia, when in fact they were DLB

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Chapter 2

Lewy Body Dementia Diagnosis - Methods

2.1 Introduction

This chapter describes the method used to undertake a study of the clinical diagnostic rates of dementia with Lewy bodies (DLB) and dementia in idiopathic Parkinson's disease (PDD) in the East Anglia region of the UK, prompted by the apparent discrepancy between the autopsy rate and clinical diagnostic rate of Lewy body dementia (LBD) discussed in Chapter 1. It will also describe a study analysing the diagnostic pathway and management of individual DLB and PDD cases, compared to non-DLB dementia cases and non-dementia idiopathic Parkinson's disease (PD) cases respectively.

2.2 Hypothesis

a) That the proportion of dementia cases diagnosed with DLB in dementia assessment services in East Anglia is lower than that expected on the basis of autopsy studies of 15-20% (Oinas *et al.*, 2007)(Perry *et al.*, 1989).

b) That a diagnosis of PDD as a proportion of all PD cases in PD and movement disorder services in the same region will be less than the reported figure of 20% (Aarsland, *et al.* 2005).

c) The diagnosis of DLB is more difficult compared to non-DLB dementia subtypes, as indicated by DLB diagnoses taking longer and requiring more clinical investigations and appointments.

d) That dementia in PD is under-diagnosed, being made late in the disease when symptoms are moderate to severe.

2.3 Methods

The study was split into two parts one focussing on DLB and the other on PDD as they are mostly seen in separate services. DLB is largely seen in memory clinics or old age psychiatry services and PDD is seen in movement disorder or PD clinics.

2.3.1 Dementia with Lewy Bodies Diagnosis Survey

To investigate the frequency of diagnosis of DLB as a proportion of all dementia cases, six services in two different trusts (Cambridgeshire and Peterborough Foundation Trust (CPFT) and Cambridge University Hospitals Foundation Trust (CUH)) within East Anglia were surveyed (see Table 2.1). These were chosen as they were representative of the services and Trusts within the East Anglia region.

Table 2.1 Clinical services investigated for DLB diagnoses

Service	Trust	Screening period
South Rural, Old Age Psychiatry (OAP)	CPFT	1/1/2013 to 30/6/2014
City, OAP	CPFT	1/1/2013 to 30/6/2014
Fenland, OAP	CPFT	1/1/2013 to 30/6/2014
Ely, OAP	CPFT	1/7/2013 to 31/12/2014
Peterborough, OAP	CPFT	1/7/2013 to 31/12/2014
Cambridge, Memory Clinic	CUH	1/7/2013 to 31/12/2014

All new cases referred and assessed within these selected services in an 18-month period (“screening period”) during 2013 and 2014, were surveyed for diagnoses made. This entailed a brief review of the medical notes of each case from that service to detect if dementia was diagnosed. If so, further demographic and diagnostic details were recorded (Table 2.2). Patients were considered to have a DLB diagnosis if their last diagnosis in the medical notes was either “probable” or “possible” DLB or mixed dementia with DLB specifically mentioned. As one of the purposes of this process was to identify patients for further analysis of diagnostic and management pathways, the screening period was shifted to a later six-month period for the second set of three sites as they were investigated six months later or more. Identification of patients earlier on in their diagnostic pathways and therefore earlier on in their disease, would ideally mean fewer patients had died or had such severe impairment that participation was deemed inappropriate by their clinician.

Table 2.2 Demographic and diagnostic data collected for each patient with dementia

Patient demographics collected
Diagnosis - dementia subtype
Age at presentation to clinic
Gender
Cognitive score (e.g. Mini Mental Test Score)
Date of initial cognitive test
Date last seen in clinic & date first seen in clinic
Prevalent or incident diagnosis
Whether deceased in screening period

2.3.2 Parkinson's Disease Dementia Diagnosis Survey

Similarly, to investigate the frequency of diagnosis of dementia as a proportion of all PD cases three PD services in three different trusts (CUH, CPFT and Norfolk and Norwich University Hospitals Foundation Trust (NNUH)) were identified to provide a sample of PD services in East Anglia (see Table 2.3).

Table 2.3 Clinical services investigated for PDD diagnoses

Service	**Trust**	**Screening period**
Addenbrooke's PD and movement disorders clinics	CUH	1/1/2014 to 30/6/2015
Elderly Medicine PD clinics	NNUH	1/1/2014 to 30/6/2015
Brookfields PD clinics	CPFT	1/1/2014 to 30/6/2015

All patients seen in those services in an 18-month period within 2014 and 2015, aged 65 and over, were surveyed for whether a PD diagnosis was made. Demographic details were then collected for such patients - see Table 2.4 for the data obtained. Patients were recorded as having PDD where the notes specifically stated "dementia" as a diagnosis, "cognitive impairment" or similar terms were not sufficient.

Table 2.4 Demographic and diagnostic data collected for each patient with PD

Patient demographics collected
Date of PD diagnosis
Age at PD diagnosis
Gender
Whether dementia diagnosed
Date of PDD diagnosis
Disease duration before dementia diagnosis
Cognitive test score
Date first seen in clinic
PD prevalent/ incident
PDD prevalent/ incident
Whether deceased in screening period

2.3.3 Diagnostic and Management Pathway Analysis for DLB and PDD

Where medical notes recorded a patient's diagnosis as DLB or, in the case of the PDD survey, where the notes stated that dementia had being diagnosed in PD, such patients were selected for further detailed analysis of their diagnosis and management pathway. Patients were not approached for consent if:

a) their clinician deemed they were unsuitable,
b) the research team deemed they were unsuitable for social reasons apparent from the medical notes,
c) their contact details were not available, or
d) they had died.

If written consent was obtained, a matched control patient was identified for that participant from the next consecutive non-DLB dementia case or non-dementia PD case (as appropriate) seen in the service who satisfied the matching criteria (see Table 2.5 for exact criteria). If the next case declined or was not suitable, the next case that matched criteria was identified, and so on until a control patient was recruited. For DLB patients, a PDD diagnosis was an exclusion criteria for control subjects. For PDD patients, treatment with rivastigmine during the screening period was an exclusion criteria for control subjects as prescription of this drug would suggest significant cognitive impairment and possible *de facto* dementia despite dementia not being formally diagnosed by the clinician in the notes hence such subjects would not be suitable control

patients. A panel of three expert clinicians reviewed the clinical data collected from the CRFs and applied consensus criteria (McKeith *et al.*, 2005; Emre *et al.*, 2007) to each consented case to validate the clinical diagnosis with the aim of excluding cases from the analysis if the LBD diagnosis were incorrect in the LBD cases or if LBD cases were identified in the controls.

Table 2.5 Matching criteria

DLB	**PDD**
Gender	Gender
Age at dementia diagnosis (+/-5 years)	Age at referral for PD symptoms (+/-5 years)
Within similar MMSE score range at dementia diagnosis: 0-9; 10-20, 21-30	

2.3.4 Recruitment

23 DLB cases were recruited. Three DLB subjects could not be matched despite an extensive search for a control subject in each case, leaving 20 matched controls. 18 PDD cases were recruited, with similarly 3 subjects unmatched, leaving 15 control subjects. Following completion of case report forms (CRFs) (as detailed below), individual participant's diagnoses were verified by two experts. One matched case for the DLB group was subsequently excluded as they were deemed by both experts to have PDD rather than AD as was diagnosed by the clinical team, hence leaving 19 control subjects (see figures 2.1 and 2.2 for the full details of participant recruitment). The experts achieved consensus and agreed with all other diagnoses.

2.3.5 Case Report Forms

CRFs were created to collect data for the in-depth medical notes review and were divided into sections as stated in Table 2.6 below.

Table 2.6 CRF description

Section	Data Collected
Patient Details	Details of the patient's diagnosis and living conditions
Clinical Features	The various clinical symptoms the patient was reported to have by clinicians
Medical History	All the reported medical history the patient was reported as having
Drug History	Entire drug history including changes
Family History	Family history as reported by patient
Physical Examination	Details of any examination findings
Investigations	All investigations carried out
Pathway to Diagnosis	Details of clinic appointments and home visits pre-diagnosis
Post-Diagnostic Management	Details of clinic appointments and home visits post-diagnosis plus further referrals and management steps

2.4 Sample Size

To identify the proportion of dementia cases that had a DLB diagnosis, a diagnostic rate below 10% was expected. If the rate found were 5%, a sample size of about 600 would enable a confidence interval of between 3.3% and 6.7%, as per the calculation below:

$$x = \frac{\left(z^2 \times p(1-p)\right)}{d^2}$$

or $(1.96)^2$ x (.05(1-.05))/($.017^2$) = 631

To identify the proportion of cases of PD with dementia, a diagnostic rate below 20% was expected. If the rate found were 10%, a sample size of about 300 would enable a confidence interval of between 6.6% and 13.4%, as per the calculation below:

$(1.96)^2$ x (0.1(1-.01))/($.036^2$) = 267.

The actual number of dementia cases screened exceeded this figure as it became clear that the diagnostic rate was below even the low rate of 5% that was predicted. This was to enable sufficient DLB cases to be identified for detailed analysis of their diagnosis and management. Even after identifying over 2300 dementia cases, only 23 cases of DLB

were recruited for detailed notes analysis due to the low diagnostic rate and the small number of cases that were actually eligible for recruitment (see Figure 2.1).

A higher number of PD cases were also identified than planned as there was a similar difficulty in recruitment for further detailed analysis of diagnosis and management, although not to the extent of the DLB subjects since the diagnostic rate was slightly higher for PDD (see Figure 2.2).

Figure 2.1 Recruitment diagram for DLB recruits

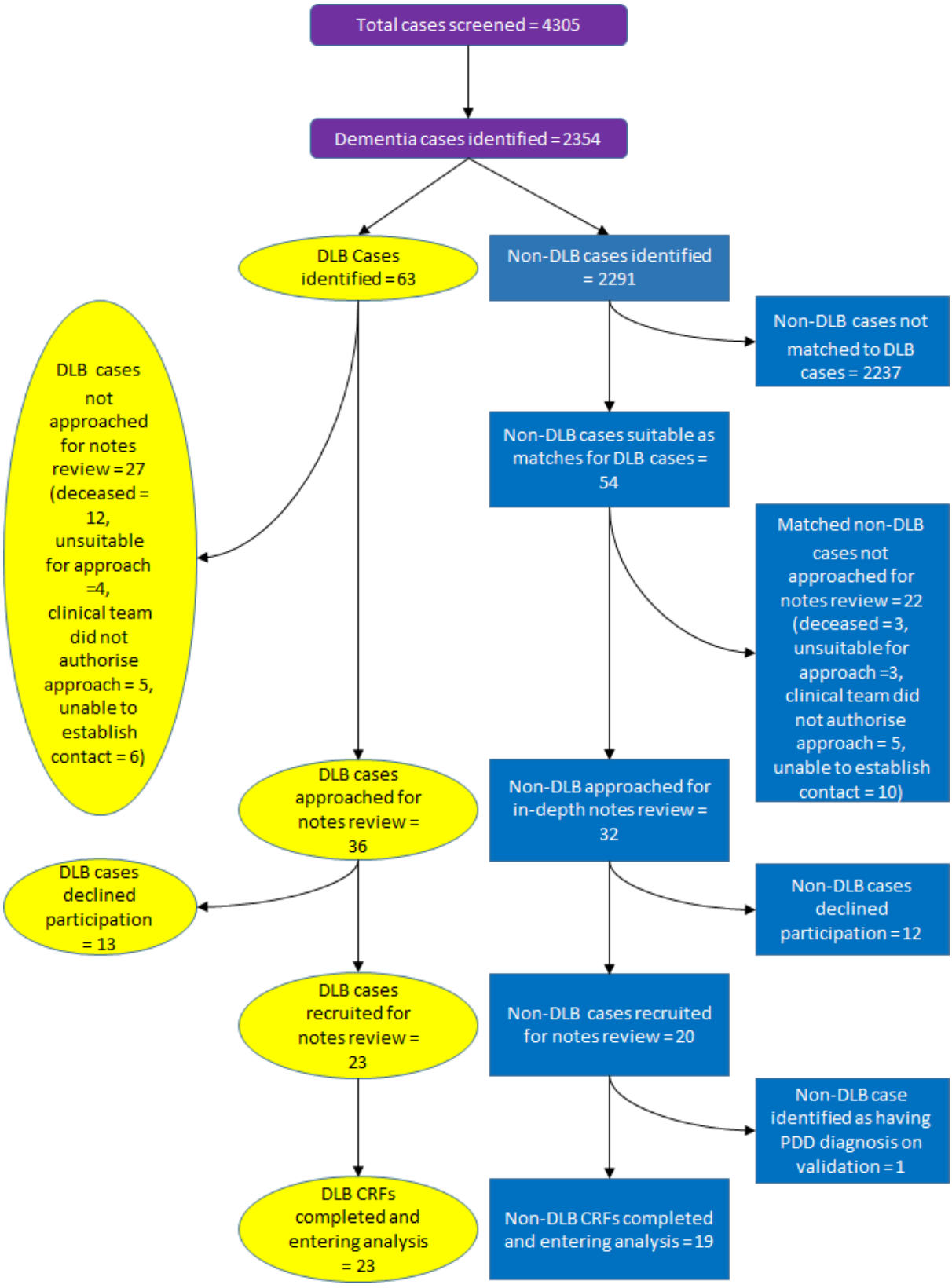
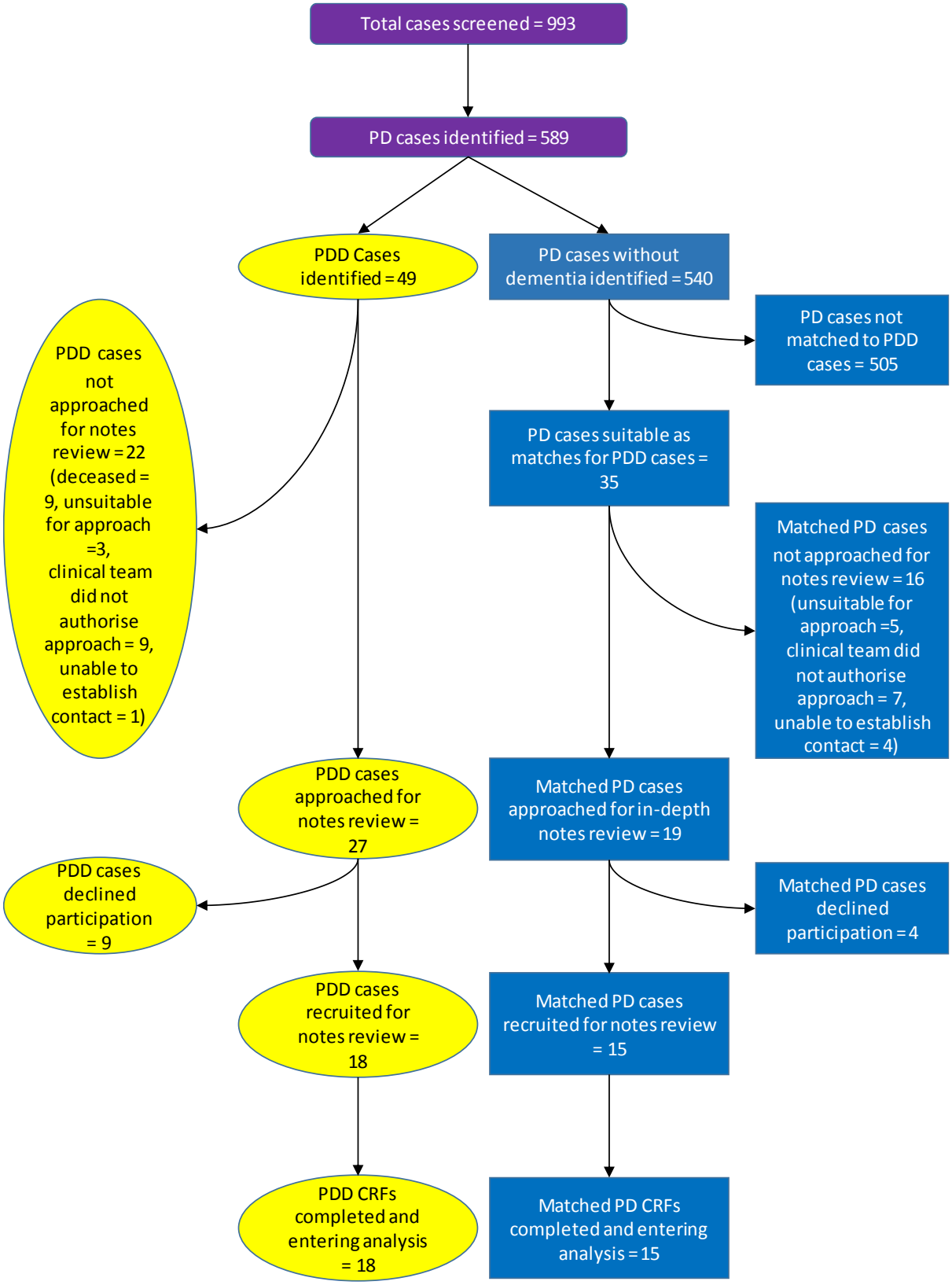


Figure 2.2 Recruitment diagram for PDD recruits



2.5 Ethics

The Confidentiality and Advisory Group provided permission to screen medical records for the surveys without obtaining patient consent. Approval was also obtained from each individual Trust (CPFT, CUH, NNUH) Research and Development (R&D) departments. Access to electronic records was also obtained at each Trust to enable the surveys. Data was collected in a standardised spreadsheet and stored on secure password protected files within each Trust's NHS server or on secure encrypted devices. For the detailed notes study, potential patients and matched controls were identified and contacted by post and provided with a patient information sheet. Following a two week period (to allow sufficient time to consider the information and to provide a written reaction if they chose), they were contacted by telephone to arrange to discuss the study in further detail. If in agreement, each patient (or carer if the patient was assessed as not having capacity to consent) signed a written consent form permitting access to their medical records for an in-depth analysis of their management and diagnosis. Appendix 3 contains an example consent form and patient information sheet for each of the two parts of the diagnostic and management pathway study.

2.6 Data Analysis

Data were analysed using the Statistical Package for the Social Sciences software version 25 (SPSS; IBM Corporation, Armonk, NY, USA). Differences in demographic and clinical data were assessed using either t-tests, analysis of variance (ANOVA), or rank-sum tests (Mann-Whitney U) as appropriate for continuous variables and $\chi 2$ test for categorical data. Correlations were carried out using Spearman's rank correlation. For each test statistic, $p<0.05$ was regarded as significant.

2.7 Funding

All studies mentioned in this chapter and chapter 3 and 4 were part of the Diamond Lewy study, funded by the NIHR (Grant Reference Number DTC-RP-PG-0311-12001).

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Chapter 3

Lewy Body Dementia Diagnosis - Results

3.1 Introduction

This chapter sets out the results from the study described in Chapter 2, starting with the diagnostic rates of both dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD) and concluding with the analysis of the diagnostic and management pathways of both conditions compared to control patients.

3.2 DLB Prevalence

1929 cases of dementia were identified, with the proportions of each subtype listed in Table 3.1. The proportion of dementia cases diagnosed with DLB was 3.3% (95% confidence interval (CI) 2.6% to 4.2%, calculated using the Wilson method). As expected, Alzheimer's dementia (AD) was the most common form of dementia diagnosed, followed by "mixed" dementia - the vast majority of which did not have any further details given, with a few where the diagnosis was explicitly stated as AD and vascular dementia.

Table 3.1 Dementia subtype proportions within the 1929 cases

Dementia Subtype	Number	Proportion (%)	95% Confidence Interval	Mean Age at Presentation	Males (%)	Mean MMSE Score at presentation	Proportion (%) deceased during Screening Period*
Alzheimer's dementia	888	46.0%	43.8 to 48.3%	81.0	34.8%	20.1	6.3%
Vascular	286	14.8%	13.3 to 16.5%	83.6	46.5%	20.0	17.5%
Mixed	455	23.6%	21.8 to 25.5%	84.4	44.2%	20.6	9.0%
Dementia with Lewy bodies	63	3.3%	2.6 to 4.2%	81.1	52.4%	20.1	15.9%
Parkinson's disease dementia	39	2.0%	1.5 to 2.8%	80.0	74.4%	20.0	25.6%
Fronto-temporal	32	1.7%	1.2 to 2.3%	69.4	56.3%	24.9	3.1%
Unspecified	166	8.6%	7.4 to 9.9%	83.5	34.9%	19.0	16.9%
	1929	100.0%		82.2	40.5%	20.3	10.2%

*These are all the deaths that were recorded in the medical notes and is the minimum figure. Other deaths may not have been recorded in the notes, hence the figure could be higher for each subtype. 95% confidence intervals were calculated using the Wilson method. MMSE = Mini mental state examination.

PDD was diagnosed in 2.0% of dementia patients seen (CI: 1.5% to 2.8%). Vascular dementia was third most common and frontotemporal dementia (FTD) was the rarest type. 8.6% of patients did not have a specified type of dementia, with clinicians simply diagnosing "dementia". Figure 3.1 shows a bar chart of the frequencies of each type.

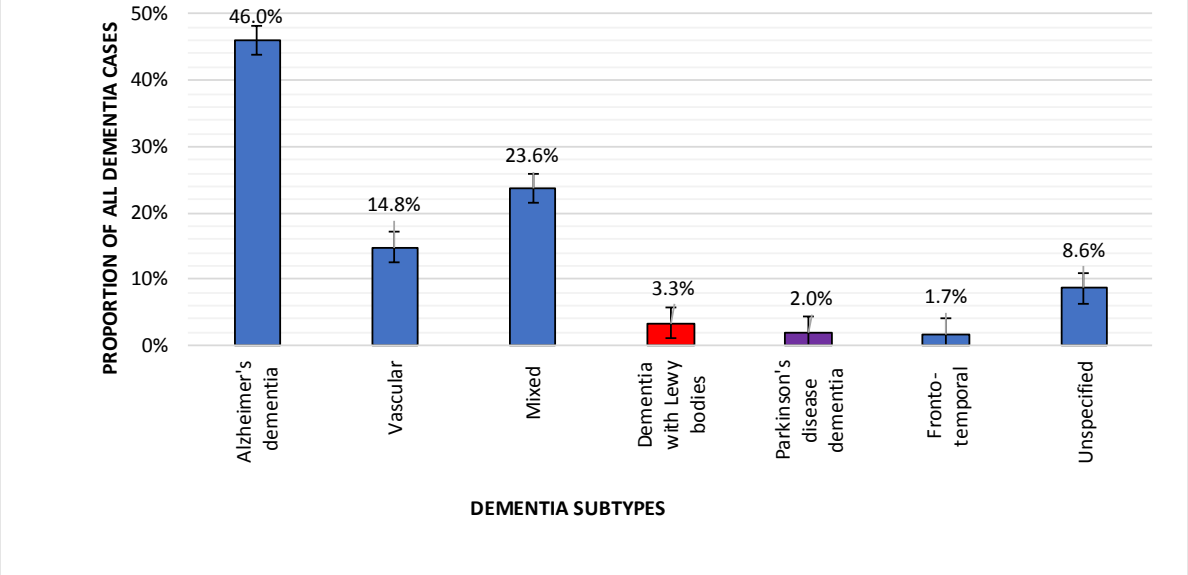

Figure 3.1 Frequency of dementia subtypes recorded. Error bars = 95% confidence intervals calculated using Wilson method

There was no significant variation in the prevalence rate across the six services (*P*=0.43, Chi Squared test) sampled (Figure 3.2) with the proportion of DLB cases ranging from 2.4% to 5.1%.

At the time of presentation to the relevant service, the mean age of patients diagnosed with DLB was 81.1. Comparison of the mean ages across groups showed significant differences (F(6,198) = 24.7, *P*= <0.001, Welch F-test/ANOVA as there was no homogeneity of variance between groups). Post-hoc analysis with the Games-Howell test revealed DLB patients were significantly older than FTD patients at presentation, but no significant differences were found between DLB and other dementia subtypes (Figures 3.3 and 3.4). FTD patients presented at a much younger age then patients of all other dementia subtypes (*P*<0.001). AD patients were also significantly younger than patients with vascular dementia (*P*<0.001), mixed dementia (*P*<0.001) or those with an unspecified dementia (*P*=0.02) at presentation to the service. Similarly PDD patients were younger than vascular dementia (*P*=0.04) and mixed dementia (*P*=0.005) patients at presentation.

The mean age of female patients with dementia was significantly higher (*P*<0.001, t-test) than males at presentation to the clinical service (Figure 3.5): 83.1 years compared to 80.9 years.

52.4% of patients with DLB were male (Figure 3.6), higher than in AD (34.8%) and vascular dementia (46.5%) but lower than in both FTD (56.3%) and PDD (74.4%).

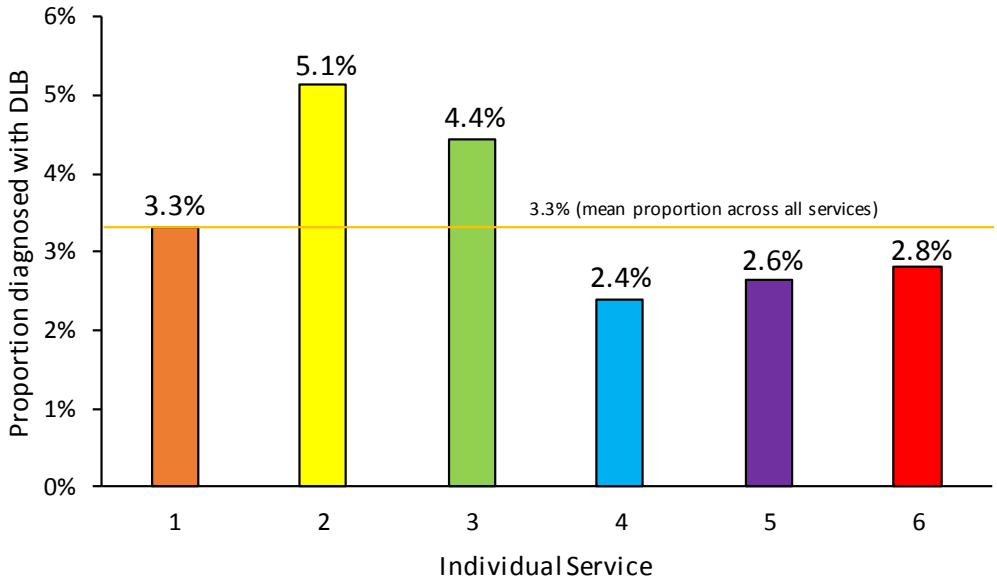

Figure 3.2 DLB prevalence for each service. No significant variation was found.

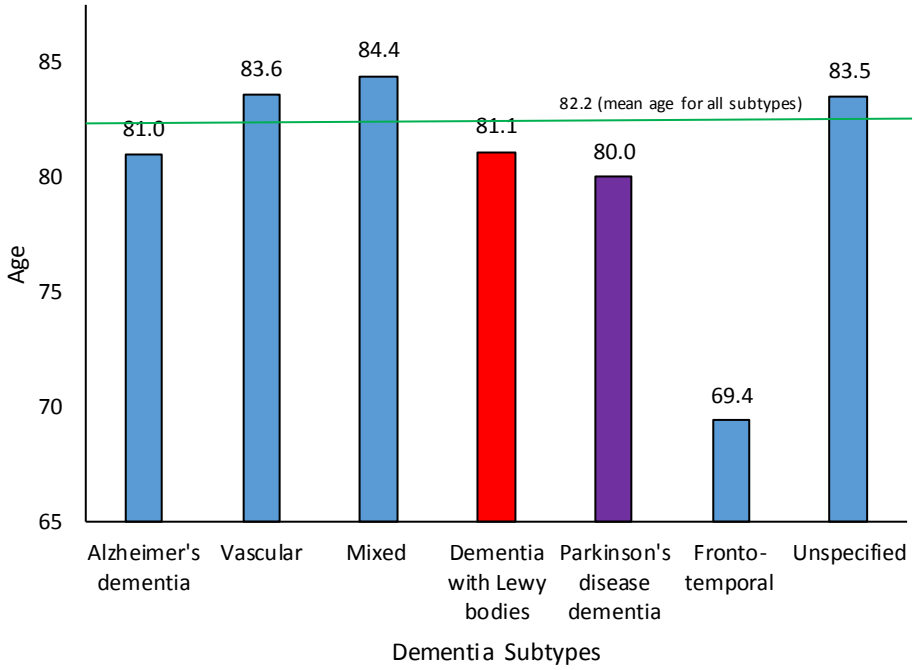

Figure 3.3 Mean age at presentation of each dementia subtype

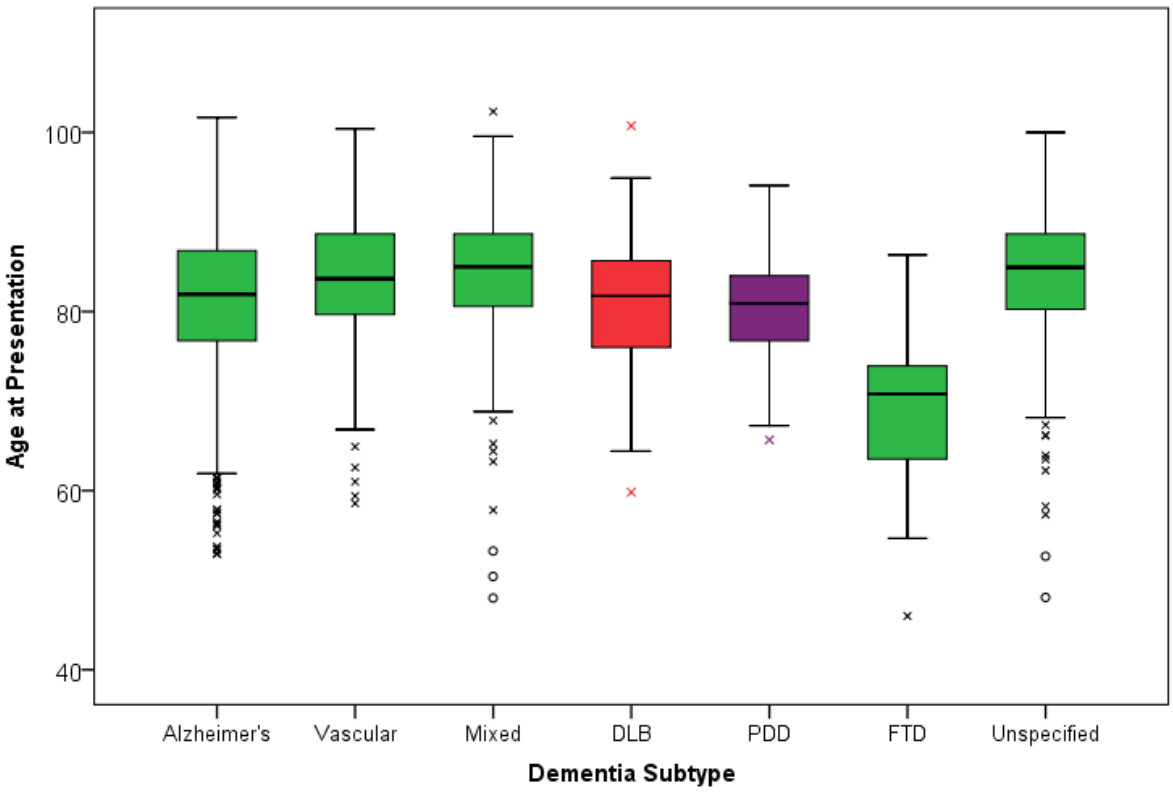

Figure 3.4 Box plots of age at presentation of each dementia subtype

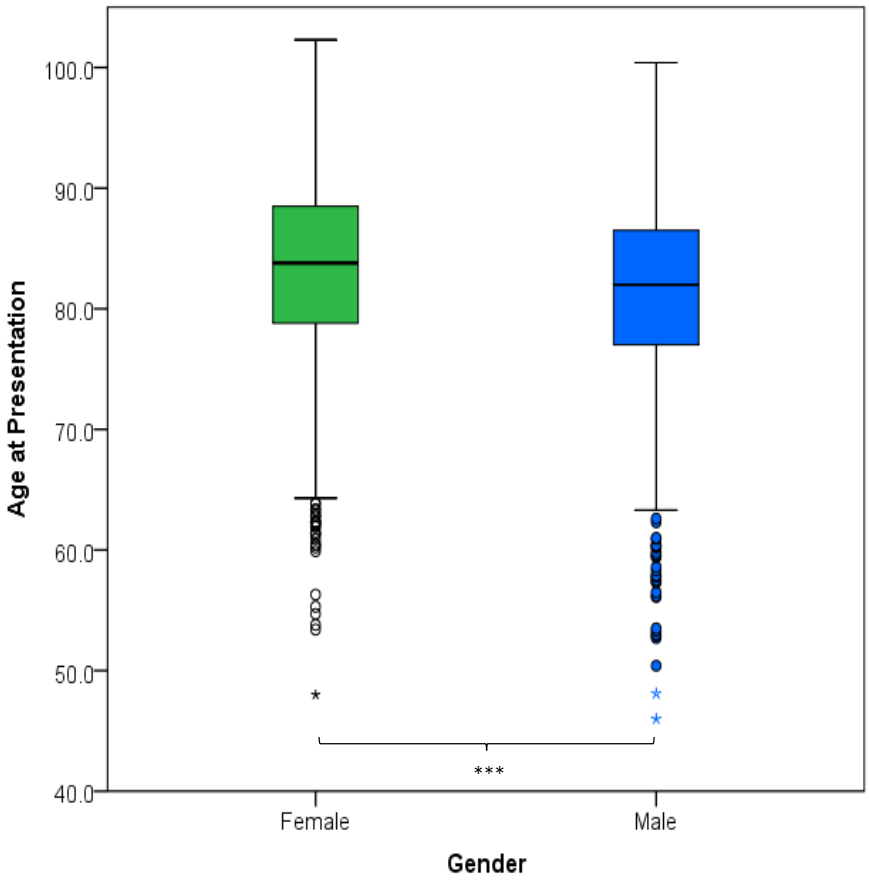

Figure 3.5 Age at presentation in years according to gender (***= *P*<0.001)

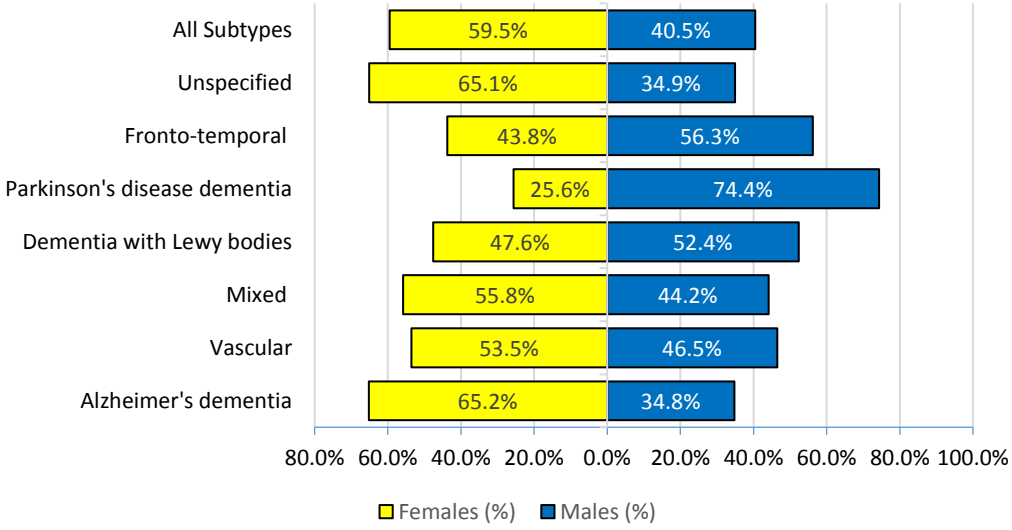

Figure 3.6 Gender distribution for each dementia subtype

3.3 PDD Prevalence

The prevalence of dementia as recorded in the medical notes of idiopathic PD cases was 8.3% (CI: 6.4% to 10.8%), with the proportion varying between services, ranging from 4.5% to 10.0% (Table 3.2). However the variation was not found to be statistically significant (chi squared test, *P*=0.11). There was no variation found in the gender distribution of patients with PD, between the services (chi squared test, *P*=0.25).

Table 3.2 PDD prevalence figures and demographics across difference services.

Service	Number	PDD number	Proportion PDD (%)	95% Confidence Interval	Mean Age at PD Diagnosis	Mean disease duration (yrs) before PDD	Males (%)	Died in Screening Period	Mean MMSE Scores (PD only)
1	359	36	10.0%	7.3%-13.6%	71.4	6.1	58.5%	11.4%	21.3
2	74	6	8.1%	3.8%-16.6%	70.8	5.3	52.7%	9.5%	-
3	156	7	4.5%	2.2%-9.0%	78.3	5.4	51.0%	-	28.0
All	589	49	8.3%	6.4%-10.8%	73.1	5.9	55.8%	11.1%	25.1

CIs were calculated using the Wilson method. Mortality figures were unavailable for service 3.

The mean age at PD diagnosis was however significantly higher in one service compared to the others, with a mean age of 78.3 years compared to 71.4 and 70.8 (ANOVA, $F(2;557) = 40.4$, $P<0.001$). The same service had the lowest rate of PDD diagnosis at 4.5% but the mean disease duration (the time between PD diagnosis and PDD diagnosis) was similar to the other services at a mean of 5.4 years (ANOVA, $F(2,35) = 0.118$, $P=0.89$). There was no mortality data (the percentage of all patients to have deceased during the screening period) available for this service, but the combined mortality rate was 11.1% for the remaining two services and was not significantly different between each (chi squared test, *P*=0.63).

Different cognitive tests were used by the services. Service 1 used a combination of Addenbrooke's Cognitive Exam – Revised 2005 (ACE-R) and Mini Mental State Examination (MMSE) and service 3 used a combination of MMSE and Montreal Cognitive Assessment (MOCA). Service 2 did not record test results, suggesting no formal tests of cognition were carried out as part of that clinic. A comparison of MMSE

scores between service 1 and service 3 in PD patients without dementia showed significantly higher scores in service 3: 28.0 v 24.0 (Mann-Whitney U, *P*<0.001).

Table 3.3 shows a comparison of the age at diagnosis, gender and mortality of PD patients with and without dementia. There was significantly more male patients (71.4%) with dementia (chi squared test, *P*=0.02) than females. Both groups had a similar mortality rate (Fisher's Exact Test, *P*=0.37) and near identical ages at diagnosis of PD. However it was not possible to detect whether patients in service 3 had died as this was not recorded in their paper medical notes – the other services used electronic records which recorded whether a patient was deceased by reference to the NHS spine.

Table 3.3 Comparison of PD and PDD patients from all three services

Diagnosis	Number	Mean Age at PD Diagnosis	%Died in Screening Period	Males (%)
PD	540	73.1	9.9%	54.4%
PDD	49	73.0	11.9%	71.4%
All	589	73.1	11.1%	55.8%

Note: mortality data was not available for service 3.

There was a significant negative correlation between the age of diagnosis of PD and the time before the diagnosis of dementia in PDD patients (Spearman's correlation, *P*=0.003, ρ= -0.47) as seen in Figure 3.7.

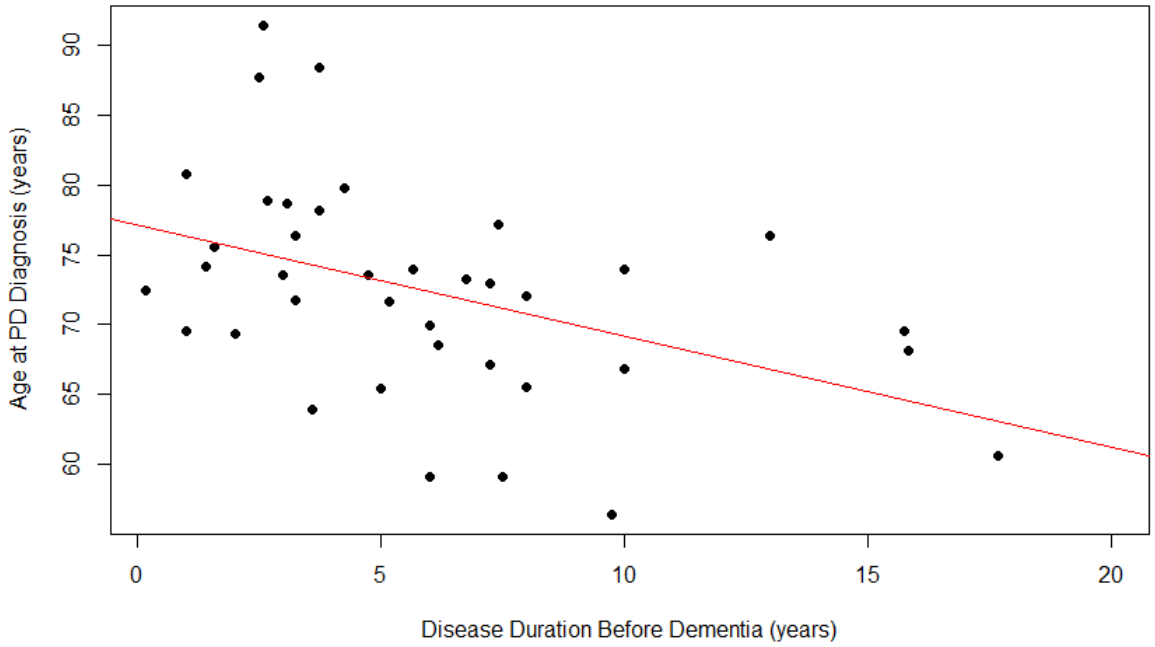

Figure 3.7 Correlation between age at PD diagnosis and disease duration before dementia (*P*=0.003, Spearman's Rho).

3.4 Summary of Prevalence Results

The proportion of dementia cases diagnosed with DLB was 3.3% and Alzheimer's disease, was the commonest form of dementia diagnosed, at 46.0%. DLB patients were found to be significantly older than FTD patients at presentation but there were no significant age differences at presentation with other dementia subtypes. Also, a higher percentage of DLB subjects were male compared to AD and vascular dementia subjects.

The proportion of PD subjects diagnosed with dementia was 8.3% and significantly more patients with PDD were male (71.4%) than female. There was also a significant negative correlation between the age at diagnosis of PD and the time before the diagnosis of dementia.

3.5 DLB Diagnostic and Management Pathway

3.5.1 Demographics

Twenty three patients diagnosed with DLB and 19 patients diagnosed with a dementia subtype other than DLB or PDD were recruited for analysis of their diagnostic and management pathway. The demographics of the recruits in the two groups are shown in Table 3.4 and revealed no significant differences in age at dementia diagnosis, MMSE score at diagnosis or gender. The control group consisted of the following subtypes of dementia: 13 AD, 5 mixed AD and vascular and 1 vascular. The matching process is further described in Chapter 2.

Table 3.4 Demographics of recruits to DLB diagnostic pathway analysis

Demographic	DLB	Non-DLB	Group Difference
Gender: males/females	14/9	11/8	ChiSq=0.04; p=0.85
Age in years: mean +/-SD	80.3 +/- 9.4	78.0 +/- 9.2	t=0.81; p=0.43
MMSE: mean +/- SD	20.2 +/- 5.5	19.3 +/-5.3	t=0.57; p=0.57

Both the age and MMSE scores are at the time of any dementia diagnosis. Chi Sq = Chi Squared statistical test.

3.5.2 Comparison of Diagnostic Pathways

Comparisons were made between the two groups with respect to the number of diagnoses made prior to their last diagnosis, the number of home visits and clinic appointments required before their final diagnosis was made, as well as the time period between the final diagnosis being made and (i) their first referral to the service, and (ii) their first appointment at the service, both for their cognitive complaint. Additional comparisons were also made between the number of clinic appointments and home visits made after their final diagnosis. The results are shown in Table 3.5.

The comparison between groups showed that before a final diagnosis was made, DLB participants on average had more alternate diagnoses, home visits and clinic appointments. They also had a longer time period on average until their final diagnosis was made from both (i) the date of referral to a service and (ii) the time seen in the service for the first time. Of these, the number of dementia diagnoses before the final

diagnosis (*P*=0.007) and also the number of clinic appointments faced by DLB patients (*P*=0.03) before a final DLB diagnosis was made, were statistically significant (with the Mann-Whitney U test) between the groups.

After a final diagnosis was made, the number of home visits faced by both groups was similar, but DLB patients had on average significantly higher clinic appointments (Mann-Whitney U, *P*=0.04).

Table 3.5 Comparison of the diagnostic pathways in DLB and non-DLB patients

	Group	Mean	Std Dev	p value (Mann-Whitney U)
Number of diagnoses made before final diagnosis	DLB	0.7	0.76	* p=0.007
	Non-DLB	0.2	0.50	
Home visits before final diagnosis	DLB	4.6	7.70	p=0.14
	Non-DLB	2.0	2.20	
Clinic appointments before final diagnosis	DLB	2.0	2.00	* p=0.034
	Non-DLB	0.6	0.81	
Total (home visits and clinic) appointments	DLB	5.2	7.60	p=0.050
prior to final diagnosis	Non-DLB	2.3	2.69	
Home visits after final diagnosis	DLB	7.3	6.40	p=0.75
	Non-DLB	7.1	7.30	
Clinic appointments after final diagnosis	DLB	1.4	1.40	* p=0.041
	Non-DLB	0.4	0.74	
Total (home visits and clinic) appointments	DLB	7.3	6.20	p=0.95
after final diagnosis	Non-DLB	7.4	7.10	
Time from referral to service to final	DLB	11.8	21.9	p=0.30
diagnosis (months)	Non-DLB	8.6	13.9	
Time from 1st appointment at service to final	DLB	10.0	21.8	p=0.35
diagnosis (months)	Non-DLB	7.4	14.0	

Old Age Psychiatrists made the majority of diagnoses in both groups, although DLB patients were slightly more likely to have a diagnosis made by a Neurologist (see Table 3.6).

Table 3.6 Comparison of the clinicians who made the final diagnosis in both groups

Clinician Making Diagnosis	Old Age Psychiatrist	Neurologist	Geriatrician
DLB	18	4	1
non-DLB	17	2	0

3.5.3 Comparison of Symptomatology

A comparison of the symptomatology of the groups (see Table 3.7), showed that, as expected, the core features of DLB (parkinsonism, visual hallucinations, fluctuating cognition) were all much more present in the DLB group, each at a highly significant level (chi squared test, $P \leq 0.001$). The supportive feature of rapid eye movement (REM) sleep behaviour disorder was also much more prevalent in the DLB group, though the difference was not as statistically significant (each at $P<0.05$, Fisher's test). There were no patients in either group with recorded severe neuroleptic sensitivity.

3.5.4 Diagnostic Threshold

The majority of patients (61%) exceeded the diagnostic threshold for "probable" DLB as set out in the 2005 consensus criteria (McKeith *et al.*, 2005), with 44% of these having at least three core features (see Table 3.8).

Table 3.7 Comparison of the core and suggestive features of DLB as seen in both groups at time of final diagnosis

At time of final diagnosis	Group	Present	Statistic	p value
Parkinsonism	DLB	70%	ChiSq=15	p<0.001
	Non-DLB	11%		
Visual Hallucinations	DLB	78%	ChiSq=22	p<0.001
	Non-DLB	5%		
Fluctuating Cognition	DLB	65%	ChiSq=10	p=0.001
	Non-DLB	16%		
REM sleep behaviour disorder	DLB	39%	Fishers	p=0.038
	Non-DLB	11%		

Table 3.8 Presence of core and suggestive features of DLB in DLB group at the time of diagnosis

Symptoms at Final Diagnosis	%
3 CORE +1 or more suggestive	17.4%
3 CORE	26.1%
2 CORE + 1 or more suggestive	17.4%
2 CORE	21.7%
1 CORE + 1 or more suggestive	4.3%
1 CORE	8.7%
1 or more suggestive	4.3%
0 features	0.0%
	100.0%

Table 3.9 Comparison of the imaging carried out in each group

Imaging Carried out	CT Scan	MRI	DaTscan	Refused	No Data
DLB	16	4	1	0	2
non-DLB	10	4	0	1	4

DaTscan = Dopamine transporter scan

3.5.5 Comparison of Imaging, Carer Stress and Co-morbidities

All patients underwent neuro-imaging – either CT head or MRI head, or in one DLB case – a dopamine transporter scan (DaTscan), unless they explicitly refused (Table 3.9).

Episodes of stress related to the patient's care expressed by their carer at an appointment were also compared, with DLB patients' carers experiencing more episodes on average (0.6 v 0.4), but this difference was not statistically significant (Mann Whitney U, *P*=0.26).

Co-morbidities such as repeated falls, constipation, urinary incontinence and orthostatic hypotension were more common in the DLB group, however only repeated falls were statistically significantly higher in the DLB group (Table 3.10). However, many of the non-

DLB group's notes did not state whether the clinician considered the presence of the symptoms.

Table 3.10 Comparison of co-morbid symptoms including autonomic symptoms in both groups

	Group	Number with symptom	Statistic	p value
Repeated Falls	DLB	18	ChiSq=6	p=0.016
	Non-DLB	8		
Constipation	DLB	9	ChiSq=0.9	p=0.47
	Non-DLB	4		
Urinary Incontinence	DLB	12	ChiSq=1.8	p=0.26
	Non-DLB	5		
Orthostatic Hypotension	DLB	4	Fishers	p=0.56
	Non-DLB	3		

3.6 PDD Diagnostic and Management Pathway

3.6.1 Demographics

Eighteen patients diagnosed with PDD and 15 PD patients not diagnosed with dementia (controls) were recruited for analysis of their diagnostic and management pathway. The demographics of the recruits in the two groups are shown in Table 3.11 and revealed no significant differences in age at referral for PD symptoms or gender. The matching process is further described in Chapter 2.

Table 3.11 Demographics of recruits to PDD diagnostic pathway analysis

Demographic	PDD	PD	Group Difference
Gender: males/females	15/3	13/2	Fisher's; p =0.56
Age at referral for PD symptoms: mean +/- SD	69.2 +/-16.6	71.5 +/-6.4	t=-.49; p=0.63

The age is at the time of referral for PD symptoms.

3.6.2 Functional impairment before diagnosis

Seven PDD patients (39%) were found, either at a clinic appointment or at a home visit, to have cognitive impairment that impaired their activities of daily living (ADLs) before a PDD diagnosis was made, with a mean duration of 1.5 years between the impairment and a dementia diagnosis. Six PDD subjects (33%) had impairments in two or more cognitive domains before their dementia diagnosis, with a mean duration of 0.3 years between the two events. However, only two (11%) of these patients had both: impairments in multiple cognitive domains and impaired ADLs due to their cognitive impairment, noted in the records prior to a dementia diagnosis being made.

3.6.3 Treatment before diagnosis

Five PDD patients (28%) were started on rivastigmine before a diagnosis of PDD was made (the diagnosis was made at a later clinical appointment), with a mean of 0.9 years before their diagnosis. One such patient had an intervening time period of 4.4 years prior to their diagnosis of PDD.

3.6.4 Investigations

A comparison of the two groups showed that PDD patients had significantly more cognitive tests than the PD control group (Mann-Whitney U, *P*=0.011) and a larger number of imaging tests (Mann-Whitney U, *P*=0.009) – see Table 3.12.

Table 3.12 Group comparison of assessments and carer stress events recorded

	Group	Mean	Std Dev	p value (Mann-Whitney U)
Number of imaging tests	PDD	1.7	1.36	p=0.009
	PD	0.6	0.63	
Number of cognitive assessments	PDD	2.8	1.60	p=0.011
(MOCA, MMSE, ACE-R, ACE-III)	PD	1.3	1.60	
Carer Stress Events Recorded	PDD	0.6	0.70	p=0.048
	PD	0.1	0.52	

3.6.5 Carer stress

Significantly higher numbers of carer reported stress events were recorded in the medical notes of PDD patients, compared to PD control patients (Mann-Whitney U, *P*=0.048), see Table 3.12.

3.6.6 Symptomatic PD subjects without dementia

Seven of the control PD subjects (47%) had cognitive impairment noted in their medical records, of which four (27%) had impairments in multiple cognitive domains and one separate subject (7%) was sufficiently impaired that their cognitive impairment impacted on their ADLs.

Two of the control patients (13%), both of whom had recorded cognitive impairment, had been treated with rivastigmine in the past, prior to the screening period, though they were never formally diagnosed with dementia. Note however that patients who were on treatments for cognitive impairment during the screening period were excluded from recruitment to the PD control group.

3.6.7 Clinicians making the diagnosis

Neurologists and geriatricians were found to make the PD diagnosis, however dementia diagnoses were made mostly by Old Age Psychiatrists (10 out of 18 subjects), see Table 3.13.

Table 3.13 Group comparison of the speciality making the final diagnosis

Clinician Making Final Diagnosis	Old Age Psychiatrist	Neurologist	Geriatrician	No Data
PDD	10	2	4	2
PD	0	6	9	0

3.6.8 Symptomatology

Visual hallucinations, fluctuating cognition and REM sleep behaviour disorder were all recorded at a significantly higher frequency in PDD patients compared to PD patients (see Table 3.14 and Figures 3.8-3.10).

Table 3.14 Presence of symptoms characteristic of dementia with Lewy bodies within PD and PDD subjects

Symptom	Group	Present	Statistic	p value
Visual Hallucinations	PDD	88%	ChiSq=12.5	p<0.001
	PD	27%		
Fluctuating Cognition	PDD	92%	ChiSq=18.6	p<0.001
	PD	7%		
REM sleep behaviour disorder	PDD	59%	ChiSq=3.3	p=0.07
	PD	27%		

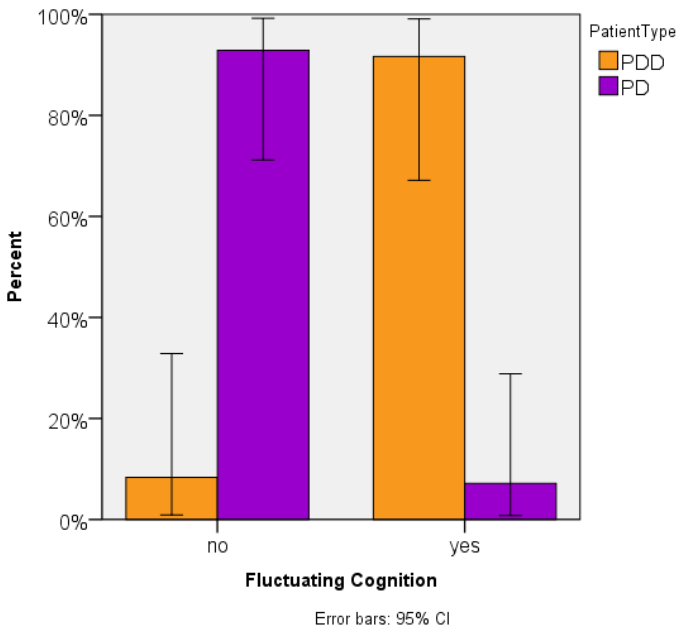

Figure 3.8 Frequency of fluctuating cognition in each group

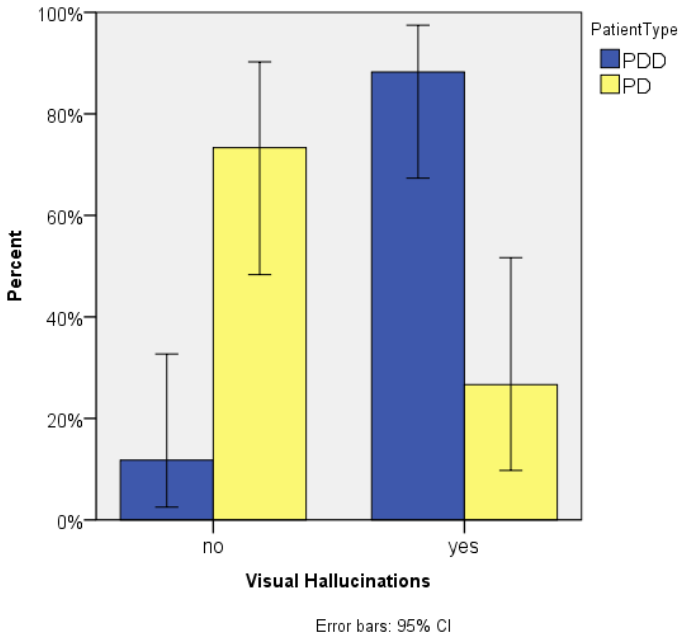

Figure 3.9 Frequency of visual hallucinations in each group

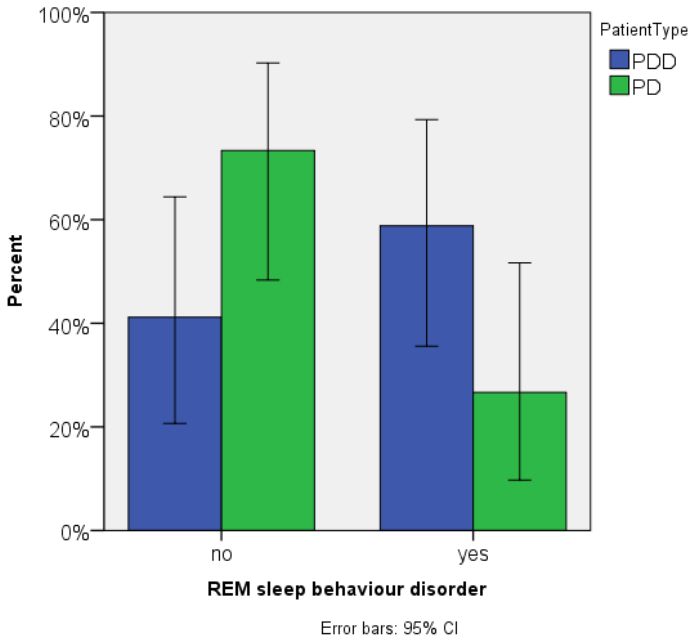

Figure 3.10 Frequency of REM sleep behaviour disorder in each group

Impairment in each of the cognitive domains was recorded at a significantly higher frequency in the PDD group (see Table 3.15). Memory impairment was ubiquitous in the PDD group and present in a third of the control group. Attentional impairment was absent

in the control group and was the least frequently impaired of all the domains in the PDD group.

Table 3.15 Presence of impairments in cognitive domains

Cognitive Domain Impaired	Group	Present	Statistic	p value
Attention	PDD	62%	Fisher's	p=0.002
	PD	0%		
Executive	PDD	85%	ChiSq=9	p=0.003
	PD	25%		
Visuo-spatial	PDD	79%	ChiSq=9.9	p=0.002
	PD	17%		
Memory	PDD	100%	Fisher's	p<0.001
	PD	33%		

Similarly, other symptoms associated with PD and LBD were recorded at significantly more frequency in the PDD group: excessive daytime sleepiness, swallowing difficulties, repeated falls, anxiety, orthostatic hypotension and changes in personality (see Table 3.16). Constipation and bladder instability was noted at similarly high levels in both groups and there was no statistical difference seen. Depression was more frequent in the PDD group, however the difference was not significant.

Table 3.16 Presence of associated symptoms

Symptom	Group	Present	Statistic	p value
Excessive Daytime Sleepiness	PDD	81%	ChiSq=7.3	p=0.007
	PD	33%		
Swallowing Difficulties	PDD	65%	ChiSq=4.6	p=0.03
	PD	27%		
Repeated Falls	PDD	77%	ChiSq=6.0	p=0.01
	PD	33%		
Anxiety	PDD	77%	ChiSq=6.0	p=0.01
	PD	33%		
Depression	PDD	59%	ChiSq=1.1	p=0.29
	PD	40%		
Orthostatic Hypotension	PDD	87%	ChiSq=11.5	p<0.001
	PD	23%		
Changes in Personality	PDD	53%	Fisher's	p=0.007
	PD	7%		
Constipation	PDD	89%	Fisher's	p=1
	PD	87%		
Bladder Instability	PDD	72%	Fisher's	p=0.41
	PD	87%		

3.7 Summary of Diagnostic and Management Pathway Results

DLB patients on average had a longer time period in secondary care, plus more home visits, and significantly more clinic appointments and alternate diagnoses, than their non-DLB dementia counterparts, before their final diagnosis was made. The majority of all dementia diagnoses, including DLB, were made by Old Age Psychiatrists.

The core features of DLB were present at much higher levels in the DLB group as would be expected; however 61% exceeded the threshold for probable DLB at the time of final diagnosis and 44% had at least three core features as per the 2005 consensus criteria.

In PDD patients, time lags were found between functional impairment, such as cognitive impairment affecting ADLs, before a dementia diagnosis was made and more than a quarter of PDD patients were started on rivastigmine before a dementia diagnosis. Cognitive impairment was also noted in several control subjects and two were started on treatment for dementia without a dementia diagnosis.

Significantly higher numbers of carer reported stress events were recorded in PDD patients' notes compared to PD patients. Visual hallucinations and fluctuating cognition

were also recorded at significantly higher frequency in PDD patients compared to PD patients.

In addition, cognitive impairment in the memory domain was present in 100% of PDD subjects and impairments in visuospatial skills, executive function and attention were also present at a significantly higher level in PDD compared to PD, as expected. Other symptoms common to PD and LBD, such as swallowing difficulties, orthostatic hypotension and repeated falls, were also found at a significantly higher level in the PDD group.

3.8 Acknowledgements

The epidemiology results presented in this chapter have been published in a paper in Alzheimer's Research and Therapy in February 2018 (Kane *et al.*, 2018) (see Appendix 1) where I was co-lead author, responsible for the data analysis and writing the manuscript and aspects of data collection.

3.9 References

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Chapter 4

Lewy Body Dementia Diagnosis - Discussion

4.1 Introduction

In this chapter the prevalence rates of dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD) identified in the results of the notes review are discussed, together with the differences found in the diagnosis and management of both compared to non-DLB dementia subjects and Parkinson's disease (PD) subjects respectively.

4.2 DLB Prevalence

DLB was diagnosed in only 3.3% (95% confidence interval (CI) 2.6% to 4.2%) of subjects diagnosed with dementia, which is lower than that found in systemic reviews of the clinical prevalence rate of DLB: 5% was reported by Hogan and colleagues (Hogan *et al.*, 2016) from combined community and secondary care studies and 7.5% was reported in secondary care populations by Vann-Jones and O'Brien (Vann Jones and O'Brien, 2014). It is much lower than that found in neuropathological studies (Fujimi *et al.*, 2008)(Jellinger and Attems, 2011), which report at least 15% of dementia patients meet pathological criteria for DLB.

This study intentionally aimed to identify frequency of diagnosis in routine clinical practice, reflecting the experience of patients being assessed in secondary care. This would likely result in lower rates than if DLB was prospectively sought with re-examination of all subjects with this in mind. The majority of other studies within the systematic reviews required the research team to assess dementia patients clinically for subtypes which could increase diagnostic rates of DLB as the research team are paying increased attention to symptomatology, particularly in studies investigating DLB prevalence specifically.

Hence the results may indicate a lower rate of disease detection, rather than true disease prevalence in the region. Differences in detection are supported by the range in prevalence of DLB observed between services (2.4% to 5.1%), though the differences were not statistically significant.

Another potential reason for lower rates than expected, is that the services studied receive mainly community based referrals and hence reflect a broader dementia population than the specialist centres often studied in secondary care prevalence papers. Services in this study were selected primarily as they were similar to psychiatry, neurology and geriatric medicine practices within the NHS throughout the UK.

Variation in true disease prevalence cannot be entirely ruled out however, the low rates seen in the region sampled could simply reflect the degree of exposure to causative or precipitating biological factors, although as yet there is no evidence of an environmental causative factor.

The prevalence of Alzheimer's dementia (AD) of 46.0% in this study was lower than found in a European wide systemic review of the prevalence of different dementia subtypes in those older than 65, at 53.7% (Lobo *et al.*, 2000) and a Japanese community study which reported 67.4% (Ikejima *et al.*, 2012). Vascular dementia prevalence here (14.8%) was consistent with that found in the studies stated (15.8% and 18.9% respectively). A UK survey of dementia prevalence commissioned by the Alzheimer's Society and based on an expert Delphi process, found AD to make up 62% of all dementia cases and vascular dementia to make up 17% (Knapp *et al.*, 2014).

However, 24% of dementia cases were found to be "mixed" in the current study and this excluded cases which specifically stated a combination of LBD with another subtype, which were recorded as DLB. In addition, 8.6% of patients were diagnosed with dementia without a subtype being given in the notes (hence classified as "unspecified"). This is a combined 32.6%, and could mean some cases of DLB (as well as other dementia subtypes) fell into these categories due to diagnostic uncertainty and could also explain a lower rate of AD than found in the prevalence studies stated. Indeed the percentage of cases recorded as mixed was much higher than reported in the aforementioned Japanese study (at 4.2%) (Ikejima *et al.*, 2012). Mixed cases were not explicitly stated in the European systematic review. It is also higher than reported by a recent report from the Alzheimer's Society – 10% (Knapp *et al.*, 2014), where "other" dementia cases were also reported to make up 3%. In addition, the study by Ikejima and colleagues (Ikejima *et al.*, 2012), reported 3.3% of cases as "other illnesses" but did not elaborate on whether these included those with an unspecified subtype of dementia. In contrast, a survey of death certificates specifying dementia in South London, reported a high level of unspecified dementia at 25.6% (Perera *et al.*, 2016), but is difficult to interpret as death certificates are often completed by doctors who have known the patient over a short admission and the diagnoses were not combined with any form of clinical assessment by the research team.

Frontotemporal dementia (FTD) patients made up 1.7% of all dementia subjects in this study, which falls between the rates found in the Japanese prevalence study of 1.1%

(Ikejima *et al.*, 2012) and the report by the Alzheimer's society, of 2%. The European study did not report FTD rates. FTD patients were also significantly younger than patients with other dementia subtypes but an average of 69 is still older than the typical mean age of presentation of FTD patients, which is in their 50s (Woollacott and Rohrer, 2016), however the higher age is likely to be due to this study being conducted on an older sample of patients within mainly old age psychiatry services.

Female dementia patients were older at presentation than males (83.1 years compared to 80.9 years), which is consistent with a faster rate of increase in the proportion of females (compared to males) developing dementia as age increases (Van Der Flier and Scheltens, 2005). Interestingly AD patients were younger at presentation than their vascular dementia counterparts, as well as those with mixed and unspecified dementia. AD prevalence is typically reported to increase steeply with age, more so than vascular dementia (Van Der Flier and Scheltens, 2005). The older age of presentation of the mixed and unspecified dementia patients adds support for some AD patients to have fallen within these two non-specific groups.

In summary, DLB prevalence was lower than reported in clinical prevalence studies, which are in turn lower than that reported in pathological studies. This study looked at routine clinical practice, whereas the majority of other studies within the systematic reviews required the research team to assess dementia patients clinically for subtypes and hence points to poor detection of DLB in clinical practice and also indicates a clear need for future work to address ways this could be increased. The subsequent diagnostic pathway analysis, which tries to understand other possible causes, is discussed further in this chapter. In addition, the large group of mixed and unspecified dementias within this study would be consistent with DLB patients (and to an extent AD patients) being given these diagnoses which are associated with uncertainty (the unspecified group more so than the mixed group) and lowering the figure specifically diagnosed with both DLB and AD subtypes.

4.3 PDD Prevalence

Dementia was diagnosed in only 8.3% (CI 6.4% to 10.8%) of patients with Parkinson's disease , much lower than the figure reported in clinical prevalence studies of between 20-30% (Aarsland, Zaccai and Brayne, 2005) (Wang *et al.*, 2014). Whilst there was some variation between the three services that were reviewed, the differences were not significant statistically.

Methodological differences are likely to have been the most significant factor in the lower rate of dementia found. The majority of recent studies aiming to ascertain the proportion of PD patients with dementia were clinic based and used a form of clinical assessment - see Chapter one. The systematic review by Aarsland and colleagues in 2005, only included papers where the study team carried out a prospective clinical examination. There was however one recent study which used similar methodology – a retrospective notes analysis, but in an Italian regional movement disorders clinic, and this showed a similar low rate of dementia in PD of 12% (Cereda *et al.*, 2016). The differences seen here may indicate that routine clinical practice does not reflect the 'true' prevalence of dementia in PD.

This study also shows service variation. One service (service 3) had a significantly higher age at diagnosis of PD at 78 (compared to 71 in the other two) and also a lower rate of dementia diagnosis at 4.5% (compared to 8.1% and 10%) though the latter was not significant. The length of time between the diagnosis of PD and the subsequent dementia was however similar to the other services. All three services recorded a mean of between 5.3 and 6.1 years. This suggests that the older age at presentation likely reflects an older population within that clinic rather than clinicians diagnosing PD later in the disease course - service 3 was a geriatrics movement disorders clinic, whereas the other two were combined neurology and geriatrics movement disorder clinics. An older population could also mean that fewer patients survived long enough for a dementia diagnosis leading to the lower prevalence of dementia in that service, but this could not be verified as mortality data was not available for this service.

Mini mental state examination (MMSE) scores were the only tests that were used in more than one service and hence comparable between services. A comparison between services 1 and 3 showed service 3 patients had significantly higher scores at dementia diagnosis (28 v 24). As stated, the lack of difference in disease duration between PD and dementia in this service, suggests that the higher cognitive scores are unlikely to represent clinicians having a lower threshold for dementia in that service, a point

supported by the lower rate of dementia diagnosis in this service compared to service 1, but it may mean that other markers of dementia were being used to make the diagnosis.

A higher proportion of patients with dementia were male (71%) than female. PD is thought to be more common in males than females, with meta-analysis of both incidence and prevalence studies of PD reporting evidence of higher rates in men (Twelves *et al.*, 2003; Pringsheim *et al.*, 2014), a 2:1 male predominance has been reported in one large study (Baldereschi *et al.*, 2000). A higher incidence of dementia in males with PD has also been found of 62.7% (Cereda *et al.*, 2016), with male gender thought to be a risk factor for dementia overall due to hormonal differences, as oestrogen is thought to be protective (Vest and Pike, 2013). However, it is far from clear that this interesting observation will translate into clinical benefit, as there is no evidence that hormone replacement therapy protects against dementia. Indeed, the largest randomised control trial undertaken thus far actually showed an increase in dementia incidence with therapy (Uchoa, Moser and Pike, 2016).

The results also showed a significant correlation between age at PD diagnosis and time before dementia, which is consistent with age being a very well established risk factor for dementia (Van Der Flier and Scheltens, 2005). A tendency for clinicians to diagnose dementia more readily in older patients cannot be excluded however, though the opposite could also be argued as older patients are far more likely to be diagnosed initially with a severe dementia, which clinicians have failed to diagnose in its earlier stages.

In summary, dementia was diagnosed in this study of clinical practice at much lower levels than other studies using clinical assessments by the research team, suggesting that either an inability by clinicians to diagnose dementia or perhaps a reluctance to do so. The subsequent diagnostic pathway analysis tries to understand which of these scenarios is more likely and is discussed further in this chapter.

Strengths of this DLB and PDD prevalence study include the large sample size (>5000 cases sampled) compared to previous studies, its representativeness, in that access to all cases within a service was allowed and, since clinical diagnoses were used, its clinical relevance. A potential limitation was the inability to verify the diagnoses which would have required full clinical examination of all cases and was therefore not feasible. However, cases were reviewed with respect to diagnosis by an expert panel, for the purposes of the subsequent in-depth notes study, and a diagnosis of LBD was validated in each case recruited as such. This provides a degree of confidence that those patients

in whom LBD was diagnosed in the prevalence study, are likely to have a diagnosis of LBD. One participant recruited as a control for the DLB diagnostic pathway study, as he was diagnosed with AD by the clinical team, was however considered to have PDD by the panel, and therefore excluded from the analysis. This is also consistent with the notion that LBD cases are being misdiagnosed as other dementia subtypes.

4.4 DLB Diagnostic Pathway

DLB patients needed a significantly greater number of clinic appointments and had significantly more diagnoses before their final diagnosis than patients with other subtypes of dementia. The mean number of home visits before final diagnosis and the total number of appointments (clinic attendance and home visits) were also higher for DLB patients but these differences were not statistically significant. A higher number of both clinical contacts before diagnosis and alternate diagnoses, would suggest difficulty on the part of clinicians to reach the diagnosis. The mean times experienced by patients from both referral to the secondary care service and their first appointment in that service, to the establishment of a final diagnosis, were also higher for those with DLB, but these differences were again not statistically significant. Post diagnosis, the number of clinic appointments attended by DLB patients was also higher than non-DLB patients. Home visits were however similar in number.

The results of this study are consistent with the retrospective study by Galvin and colleagues (Galvin *et al.*, 2010) of caregiver experience of patients with LBD, which found that two-thirds of patients saw more than 3 doctors before an LBD diagnosis was made and a third needed more than six clinic visits. In 78% of cases, a diagnosis of another disorder was made first (39% with another form of parkinsonism, 26% with AD and 24% with a primary psychiatric disorder). However the Galvin study was entirely based on caregiver perception with no independent observations or objective information to verify the diagnostic pathway. It could also be subject to recall bias especially where patient's experience is mostly negative. In addition, the study did not differentiate DLB from PDD, the latter diagnosis, in theory, should be more straightforward than a DLB diagnosis, with dementia presenting on the background of established PD. In contrast, the current study used objective information collected from medical notes written contemporaneously.

The majority of all diagnoses were made by psychiatrists, not a surprising finding as the study was mainly conducted in psychiatry services. Some patients were seen by

neurologists, who were a smaller proportion of the clinicians within the services. Psychiatrists were responsible for a similar number of DLB (78%) and non-DLB (89%) diagnoses, as were neurologists (17% of DLB diagnoses and 11% of non-DLB diagnoses). For comparison, in the study by Galvin *et al.,* 62% of LBD diagnoses were made by neurologists and only 9% by psychiatrists, the differences may simply reflect that the current study surveyed mainly Old Age Psychiatry services.

There were, as expected, significantly more core features (parkinsonism (70%), visual hallucinations (78%) and fluctuating cognition (65%)) in the DLB group than the non-DLB group (11%, 5%, and 16% respectively). REM sleep behavior disorder (RBD) was also significantly more common in the DLB group, but to a lesser extent (39% v 11%). The latter could be due to clinicians not asking about the symptom or patients and carers not reporting or being aware of the typical features of this condition.

At the time of diagnosis, 61% of patients diagnosed with DLB had more clinical features than required by the criteria to meet the diagnostic threshold for “probable” DLB as set out in the 2005 criteria (McKeith *et al.*, 2005), with 41% having all three core criteria or all three core criteria plus a suggestive feature, which would be in line with clinicians needing to feel very confident about a DLB diagnosis before assigning it to patients. Very few patients were diagnosed based just on two core criteria (21%) or just one core and one suggestive (4%), both of which would satisfy the criteria for “probable” DLB. There were 12% however who only had sufficient diagnostic features for “possible” DLB. Interestingly only one DLB case had a dopamine uptake scan (DaTScan), and in this subject the scan was abnormal. A DaTScan or Dopamine SPECT (Single-photon emission computed tomography) is an imaging test shown using autopsy validation to have good sensitivity (>80%) and specificity (>90%) for DLB (Walker and Walker, 2009; Thomas *et al.*, 2017). An abnormal DaTScan is a suggestive feature in the 2005 criteria (McKeith *et al.*, 2005) and an indicative biomarker in the updated 2017 criteria (McKeith *et al.*, 2017), which is discussed below. The low rate of use of the scan may be due to lack of funding or lack of availability for the services studied. An abnormal scan may have increased the likelihood of clinicians diagnosing “probable” DLB, as just one more criteria is required: a single core feature.

The higher threshold that appears to be set by clinicians is despite the excellent specificity of the diagnostic criteria. Intriguingly, the 2005 criteria have lower sensitivity (32%) than specificity (98%) in diagnosing DLB according to the largest study to use autopsy data to assess the 2005 criteria, with 2868 cases (Peter T. Nelson *et al.*, 2010)

(Huang and Halliday, 2013). This suggests that setting a higher threshold than the 2005 consensus criteria is unlikely to increase the diagnostic accuracy, but would reduce the numbers detected even further. Hence the high threshold used by clinicians may well explain the relatively low proportion of DLB cases identified in this study.

A recent pooled meta-analysis of DLB diagnosis accuracy showed 20% of DLB patients were diagnosed incorrectly as DLB (Rizzo *et al.*, 2017), however that review (in the case of the 2005 criteria) only looked at studies where the criteria were applied to late stage (severe) DLB patients, and in this group sensitivity was increased to 88% but specificity fell to 80%. The latter is consistent with increasing numbers of late stage AD patients developing hallucinations, fluctuations and parkinsonism (Peter T Nelson *et al.*, 2010).

A lack of a viable biomarker may be hindering clinicians in the diagnosis of DLB and leading them to use higher thresholds for symptomatology. This study shows that despite DaTScans being a very accurate and hence useful determinant of DLB pathology, they are not being used widely. A more accessible biomarker, which is also sensitive, may narrow the gap between clinical and pathological prevalence rates.

The importance of making a DLB diagnosis is underlined by the experiences of patients and carers. This study showed that DLB patients' carers experienced more stress than carers of non-DLB dementia patients (though this was not statistically significant). There were also higher levels of co-morbidities in DLB patients, who had a higher rate of falls. Other symptoms of constipation, urinary incontinence and orthostatic hypotension were also more common in the DLB group, but these differences were not statistically significant.

High levels of care giver stress have been identified in LBD previously and this was associated with behavioural problems, impaired activities of daily living and isolation (Leggett *et al.*, 2011), though that study did not compare DLB with other dementia subtypes. A review by Zweig and Galvin (Zweig and Galvin, 2014) looked in more detail at the experiences of patients and carers and identified far-reaching consequences of having LBD that may not be appreciated without a diagnosis being made. The gravest danger being the inadvertent use of neuroleptics, which can be fatal in DLB if neuroleptic malignant syndrome is triggered, and can more commonly lead to worsening of their debilitating movement disorder, something which may not even be realised by doctors or patients if thought of as a natural deterioration of their dementia.

LBD patients also report lower quality of life scores than AD patients, often due to autonomic and neuropsychiatric symptoms that are common in LBD. Higher care giver stress was also reported in DLB patients compared to AD patients, in association with delusions, hallucinations, anxiety and apathy in DLB patients. The multitude of symptoms can complicate the provision of clinical care for clinicians who may not appreciate which symptoms are most troubling and hence need addressing. There is also a suggestion of a higher mortality risk of DLB than AD (Zweig and Galvin, 2014). In addition, if parkinsonism is not recognized, patients may not receive beneficial symptomatic treatment for bradykinesia or rigidity.

Hence making the diagnosis of DLB is an important step in appreciating the potential complications from the condition and for providing the necessary level of support and clinical care to the patient and their care giver.

Newer criteria to try and improve the diagnosis have been published since this study was conducted (McKeith *et al.*, 2017), see Table 4.1. RBD has been upgraded to a core criteria and both polysomnography for detecting RBD and MIBG (^{123}Iodine-metaiodobenzylguanidine myocardial scintigraphy) scanning for detecting cardiac sympathetic nerve loss, have become indicative biomarkers. Neuroleptic sensitivity is no longer part of the criteria. Whilst the addition of two new biomarkers will make it easier for clinicians to make the diagnosis where core clinical features are not present, both scans have their difficulties. MIBG scans for example are popular in Japan but in the UK their use is limited and they are not always available, though this may change if the new draft National Institute for Health and Care Excellence guidelines for dementia, which recommend their use, are accepted (National Institute for Health and Clinical Excellence, 2018). In the case of polysomnography, it would currently be impracticable to refer everyone suspected of DLB for an overnight sleep study – as it would overwhelm the limited services available. Patients would need to be carefully selected, for example where there is a suspicion but the history is not clear cut and if an abnormal study would assist in making the DLB diagnosis from “possible” to “probable” - say if the patient only had one core feature such as visual hallucinations and no other features. In addition, such patients could be identified with a careful history from their partner without the need for a sleep study, but on the flip side this is inherently inaccurate as it is based on a subjective experience. Hence, whilst RBD and polysomnography for its detection are proven to be accurate biomarkers, they both have their limitations in terms of usefulness. This also highlights another difficulty with the criteria as they stand: –a patient with only parkinsonism and an abnormal DaTScan will fall into the “probable” DLB category,

similarly those with only RBD and an abnormal sleep study. However, from a clinical perspective they represent only one clinical abnormality in each case, and using them twice is likely to diminish diagnostic accuracy.

Hence in summary there is a need for a simple biomarker that is easily available and easy to conduct. If such a biomarker were also sensitive, this would be a major step in narrowing the difference in clinically diagnosed and pathologically prevalent rates of DLB. A recent study suggested phosphorylated alpha-synuclein in autonomic nerves supplying the skin could indeed be such a biomarker, proving to be sensitive and specific for DLB (Donadio *et al.*, 2017). Whilst the study shows promise, it was limited to very few non-autopsy confirmed subjects (18 DLB, 13 non-DLB dementia and 25 healthy controls), and therefore requires further validation, and is not close to being introduced into routine clinical practice.

The lack of an accessible sensitive biomarker leads to a reciprocal cause and effect as it means a difficulty in recruiting DLB patients, as many are misdiagnosed, hence they often do not enter research trials that are trying to identify a viable biomarker for such patients, meaning a perpetuation of this issue.

Another factor that may lead to fewer DLB diagnoses, is the perceived lack of specific treatments for DLB. The current cognitive therapies for DLB of donepezil, rivastigmine, galantamine and memantine are the same treatments given to AD patients (Stinton *et al.*, 2015) (O'Brien *et al.*, 2017). Hence clinicians may be less motivated to differentiate the two disorders, where the clinical history has overlapping features. This simplistic approach overlooks the additional complications (movement disorders, autonomic, psychiatric and neuroleptic related) that are associated with DLB, but nevertheless may be a factor in the gap between clinical and neuropathological rates of DLB. Whilst education and awareness of these complications is increasing, a disease specific treatment for DLB, is likely to increase the need to not miss a DLB diagnosis.

Table 4.1 The latest diagnostic criteria for DLB (McKeith *et al.*, 2017)

<table>
<tr><th colspan="3">Diagnostic Criteria</th></tr>
<tr><td colspan="3">

For a patient with dementia (defined as a progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational functions or with activities of daily living):

A “probable” DLB diagnosis requires at least two core features or one core feature and at least one indicative biomarker, whereas
A ”possible” DLB requires only one of the seven from the list of core features or indicative biomarkers.

Supportive biomarkers are helpful in making the diagnosis but their specificity to DLB is not clear.
In addition, a patient must have either developed dementia before, or within one year, of the onset of parkinsonian symptoms; hence if more than a year passes before the onset of dementia following parkinsonism, the alternative diagnosis of Parkinson’s disease dementia (PDD) is made.

</td></tr>
<tr><th>Core Features</th><th>Indicative Biomarkers</th><th>Supportive Biomarkers</th></tr>
<tr>
<td>
Recurrent visual hallucinations
Fluctuating cognition
Spontaneous features of parkinsonism
Rapid eye movement (REM) sleep behaviour disorder (RBD)
</td>
<td>
Polysomnography confirming RBD by showing REM sleep without atonia
Abnormal dopamine transporter (DAT) imaging revealing reduced DAT uptake in the basal ganglia
^{123}Iodine-metaiodobenzylguanidine (MIBG) myocardial scintigraphy revealing loss of postganglionic sympathetic cardiac innervation
</td>
<td>
Relative preservation of medial temporal lobe structures on CT or MRI
Generalized low uptake on Single Photon Emission Tomography (SPECT)/ Positron Emission Tomography (PET) perfusion/metabolism scan with reduced occipital activity ± posterior cingulate island sign on Fluorodeoxyglucose (FDG)-PET imaging
Prominent posterior slow-wave activity on EEG with periodic fluctuations in the pre-alpha/ theta range
</td>
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</table>

4.5 PDD Diagnostic Pathway

The results of this study suggest that a diagnosis of dementia is often delayed in PD patients. The diagnostic criteria for PDD (Emre *et al.*, 2007) as published by the Movement Disorder Society (MDS) is shown in Table 4.2. Subsequent to its release, a follow-up paper described how to interpret the criteria (Dubois *et al.*, 2007) and suggests two levels of certainty: a lower level ("level 1") for clinicians and a higher ("level 2") for the purposes of research. The level 1 algorithm as set out by Dubois and colleagues for diagnosis is shown in Table 4.3.

Table 4.2 Clinical diagnostic criteria for PDD as published by the Movement Disorders Society (Emre *et al.*, 2007)

MDS Criteria for Diagnosis of Dementia in Parkinson's Disease
1.PD diagnosed in accordance with UK PD Brain Bank criteria
2.Dementia syndrome with insidious onset & slow progression, in the context of established PD and diagnosed by history, clinical and mental examination, defined as:
(i)Impairment in at least 2 domains (attention, executive function, visuospatial function and memory)
(ii)Representing a decline from premorbid level
(iii)Deficits severe enough to impair daily life (social, occupational, or personal care), independent of the impairment ascribable to motor or autonomic symptoms

Probable PDD requires both 1 and 2 to be present, with a clear history of dementia established in the context of motor symptoms, and no other plausible causes of dementia syndrome including systemic disease, drug intoxication, major depression or probable vascular dementia.

Table 4.3 Suggested algorithm for diagnosis of PDD (Dubois *et al.*, 2007) at level 1 that could be used by clinicians.

Level 1 Algorithm for PDD diagnosis
1. A diagnosis of Parkinson's disease based on the UK Brain Bank Criteria for PD
2. PD developed prior to the onset of dementia
3. Mini mental state examination score (MMSE) below 26
4. Cognitive deficits severe enough to impact daily living
5. Impairment in at least two of the following tests (as further described in the paper):
-Months reversed or Sevens backward (Attention)
-Lexical fluency or Clock drawing (Executive Function)
-MMSE pentagons (Visuospatial skills)
-3 Word recall (Memory)

Seven of the 18 PDD patients recruited to this study were found, either at a clinic appointment or at a home visit, to have cognitive deficits that impaired daily life as measured by activities of daily living (ADLs), for a mean duration of 1.5 years, before a dementia diagnosis was made. Six PDD subjects had impairments in two or more cognitive domains before their dementia diagnosis, with a mean duration of 0.3 years between the two events. Two of these patients had both: impairments in multiple cognitive domains and impaired ADLs due to their cognitive impairment, noted in their records prior to a dementia diagnosis being made. This suggests that there was a lag between satisfaction of the dementia criteria and the formal diagnosis.

Another five PDD patients were being treated with Rivastigmine before a diagnosis of dementia. The mean length of time before the diagnosis was made in these patients was 0.9 years. One of these patients (but none of the other four) had evidence of psychosis prior to the onset of dementia, and for this patient, rivastigmine was started at the same time as the onset of these psychotic symptoms. Visual hallucinations were present in two of the other four patients prior to dementia and to the commencement of rivastigmine, and it is possible they were started on rivastigmine for visual hallucinations. Rivastigmine is licenced only for the treatment of dementia and hence its use for these indications would be “off-licence”.

The results from the diagnostic study also suggest that some PD patients that fit the criteria for dementia in PD, were not being diagnosed with dementia. Four of the 15 PD subjects in the control group had cognitive impairment noted in their medical records in multiple cognitive domains. One separate subject was sufficiently cognitively impaired for their ADLs to be affected. Two of the control patients, both of whom had recorded cognitive impairment, had been treated with rivastigmine in the past, prior to the screening period, though they were never formally diagnosed with dementia. Neither patient had evidence of psychosis or visual hallucinations. The study actively excluded patients who were on rivastigmine during the screening period, from the control group, hence the number of patients on rivastigmine without a dementia diagnosis in these services is likely to be higher.

Interestingly, whilst geriatricians and neurologists made the initial PD diagnosis, a dementia diagnosis was made in the main by old age psychiatrists (in 10 out of 16 PDD patients). PD patients are regularly followed up by neurologists and geriatricians (as well as specialist nurses), who would be able to make a dementia diagnosis, but the results

suggest that most PD patients were referred to psychiatry services for diagnosis of PDD. As noted earlier, DLB diagnoses were also made by psychiatrists, though most DLB patients were seen by psychiatrists initially. Hence, in this study LBD was made primarily by psychiatrists, irrespective of whether the initial referral to specialist services was to neurology or psychiatry.

Clinicians also used more cognitive and imaging tests with respect to PDD patients than PD patients, but as dementia is an additional condition in the PDD group, on the baseline of PD that is found in both groups, this is not unexpected.

All four of the cognitive domains that are typically impaired in PDD (attention, executive function, visuospatial skills and memory) were much more impaired in the PDD group. Memory impairment was a feature in all PDD patients, and executive dysfunction in 85%. However memory impairment and executive dysfunction was also found in more than a quarter of patients with PD. None of the PD patients were found to have attentional impairment however.

Visual hallucinations and fluctuating cognition were present significantly more in PDD (88% and 92% respectively) than PD control subjects (27% and 7%), suggesting these clinical features could be surrogate markers, used by clinicians to make a dementia diagnosis – they are not part of the MDS criteria. Both are core features of DLB, which shares many of the pathological features of PDD (Jellinger and Korczyn, 2018), hence it is possible clinicians are making the dementia diagnoses with this in mind, or perhaps from their own clinical experience that PDD is often associated with these features. Visual hallucinations are less frequent in PDD than DLB, but have been found to be a strong predictor for the onset of dementia (Anang *et al.*, 2014). In addition, fluctuations in cognition have been reported at a similar frequency in DLB and PDD patients but were not found at all in PD patients (Ballard *et al.*, 2002).

RBD was also found at higher levels in PDD compared to PD patients (59% v 27%) but the difference was not significant. RBD is also reported to be a strong predictor for dementia in PD subjects (Anang *et al.*, 2014).

The implications of a dementia diagnosis on the patient and carer can also be seen within the results with increased orthostatic hypotension and psychiatric features (anxiety and changes in personality) in PDD patients compared to those without dementia.

Swallowing difficulties, repeated falls and excessive daytime somnolence were also more frequent in the PDD group. The increase in carer stress in PDD compared to PD patients, could easily be explained by these differences and subsequent increase in care burden.

Hence overall, the results suggest a delay in diagnosis of dementia in some PD patients, where clinically the patient has features consistent with dementia, but they are not diagnosed promptly, only later on in the disease course, if at all. This may lead to a lower rate of diagnosis clinically and explain the low proportion of patients with dementia found in the earlier prevalence study. The longitudinal study by Hely and colleagues (Hely *et al.*, 2008) which observed PD patients over 20 years from diagnosis, found 83% of survivors developed dementia and 75% who had not survived to 20 years also developed dementia before they died. It also found neurologists were more likely to underestimate than overestimate the prevalence of dementia in PD patients, recommending that dementia should be actively sought and excluded rather than assumed to be absent. Neurologists and geriatricians in this study appeared to defer diagnosing dementia to their psychiatry colleagues.

A lower rate of diagnosis in clinical practice has important implications for the patients and their care givers who benefit from a diagnosis being made as the consequences of its development has a profound effect on the patient and carer, as described. A diagnosis allows for the provision of support services to cater for these. Dementia, together with the increased carer stress and an increased falls risk we report here, also leads to loss of insight, impaired driving skills, poor judgement and poor financial decision making, amongst other difficulties (Aarsland *et al.*, 2001). Health care providers would also need to adapt their services to cater for a higher population of their patients experiencing the difficulties of having dementia.

Hely and colleagues suggest serial brief regular assessments throughout the disease course to detect cognitive decline as a means to ensure that diagnosis is not missed (Hely *et al.*, 2008). A simple biomarker, sensitive for dementia, in the context of PD would be another means of increasing detection. Disease modifying treatment specific for dementia in PD, similar to DLB, would also increase the vigilance by clinicians (especially neurologists and geriatricians who carry out the regular follow-ups) for its features and increase detection.

4.6 Conclusion and Next Steps

This study revealed low clinical prevalence rates of DLB and dementia in PD, which are likely to be as a result of low detection rates. In DLB, clinicians appear to be requiring a high threshold before making a diagnosis and many cases may be being missed due to diagnostic uncertainty. In PDD, there appears to be a lag in the diagnosis, beyond the onset of symptoms of dementia. Hence both subgroups of LBD patients appear to be being underdiagnosed. A sensitive, easily accessible biomarker is therefore needed as are disease modifying treatments to try and increase detection rates through making the diagnosis process easier and by increasing motivation, and hence vigilance, to make an LBD diagnosis.

One source of a potential biomarker or a possible avenue for disease modifying treatment is inflammation in association with these conditions, if inflammation was found to be increased in Lewy body dementia. It is now increasingly recognised that inflammation plays a part in the pathology of dementia (Amor *et al.*, 2014) (Lee *et al.*, 2010), though it's not clear whether that is beneficial, detrimental or both. The next part of this thesis looks at whether inflammation plays a part in LBD pathology, hence potentially providing a source of a biomarker or a route for disease modifying treatment, starting with a review of the current evidence of inflammation in LBD.

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Chapter 5

Inflammation in Lewy Body Dementia – Background Literature Review

5.1 Introduction

The etiology of dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD) remains unclear, but a role for inflammation has been proposed, extrapolating from the emerging evidence for inflammation in the etiology of Alzheimer's disease (AD) and other neurodegenerative conditions. In AD neuropathological studies report evidence of brain inflammation (McGeer and McGeer, 2013), positron emission tomography (PET) imaging reveals microglial activation *in vivo* (Hamelin *et al.*, 2016, 2018; Fan *et al.*, 2017), genetic studies implicate polymorphisms in genes involved in the inflammatory response as risk factors, epidemiological studies indicate a protective effect of non-steroidal anti-inflammatory drugs (NSAIDs) and mouse models of AD suggest NSAIDs reduce neuroinflammation and protein deposition (Lee *et al.*, 2010; Latta, Brothers and Wilcock, 2014; Morales *et al.*, 2014).

In light of the gathering evidence for neuroinflammation in AD, we reviewed the literature for evidence that inflammation also plays a role in the etiology of Lewy body dementia (LBD).

5.2 Literature Search Strategy

References were identified using searches of PubMed with key words. The following combinations were used in a search of titles and abstracts in June 2015 and updated in March 2018 (the number of articles yielded is noted in brackets):

1. 'Lewy' and ('inflammation' OR 'neuroinflammation') (186 articles)
2. ('Parkinson's disease dementia' OR 'PDD' OR 'DLB' OR ('Dementia AND Parkinson*')) AND ('neuroinflammation' OR 'inflammation') (361 articles)
3. 'synuclein' AND 'microglia' (295 articles)
4. 'synuclein' AND ('inflammation' OR 'neuroinflammation') (410 articles)

The abstracts of these articles were screened and full texts obtained of those articles which were potentially relevant to this review. In order to ensure that all relevant references were sourced, references were in turn reviewed for other relevant articles, supplemented by articles known to the authors.

5.3 Neuroinflammation

Neuroinflammation describes the response to injury within the central nervous system (CNS) leading to the activation of microglia and astrocytes, release of cytokines and chemokines, invasion of circulating immune cells and complement activation. Microglia are the resident macrophages of the CNS, originating from progenitors in the embryonic yolk sac (Ginhoux *et al.*, 2013). They provide the innate immune response to invading pathogens and also initiate the adaptive response through antigen presentation (Nayak, Roth and McGavern, 2014). Microglia are also involved in non-immunological roles, including synapse formation and maintenance.

Microglia are resting or "inactivated" under physiological conditions with characteristic ramified morphology and distributed within brain regions, such that rami are close but not touching, implying each cell has its own distinctive territory. But even in this inactive state, they have been shown using two-photon microscopy to be vigilant: continuously monitoring the extracellular spaces with their processes and protrusions in adult mice (Nimmerjahn, Kirchhoff and Helmchen, 2005). Activation leads to morphological change with microglia assuming a more rounded amoeboid shape, with targeted movement of processes towards sites of injury or stimuli to initiate phagocytosis (Nimmerjahn, Kirchhoff and Helmchen, 2005) and also leads to production of chemokines, that amplify the response by recruiting other microglia, plus cytokines, free radicals and proteases which destroy infectious organisms and infected neurons.

The potential role of microglia as primary contributors to neurodegeneration was highlighted by the discovery that null mutations of triggering receptor expressed on myeloid cells 2 (TREM2), which is only expressed in microglia within the CNS, cause Nasu-Hakola disease, a rare condition leading to a degenerative mid-life dementia, amongst other impairments (Dardiotis *et al.*, 2017). TREM2 suppresses inflammatory processes and promotes phagocytosis of cell debris and bacteria, lending support for a generally protective function (Ransohoff, 2016). TREM2 variants have been associated with increased risk of developing a number of degenerative conditions including Alzheimer's disease, frontotemporal dementia and Parkinson's disease (Yeh, Hansen and Sheng, 2017). Intriguingly, ApoE has been found to be a high affinity ligand to TREM2 and can coat apoptotic neurons to promote phagocytosis through this interaction, though there was no variation in binding based on the different isoforms of ApoE, which can alter risk of developing AD. However mutations in TREM2 associated

with AD, can block the binding between TREM2 and ApoE (irrespective of isoform) (Atagi *et al.*, 2015).

Microglia appear to have an important part both in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a neurotoxin that leads to parkinsonism) disease progression and idiopathic PD (Gao *et al.*, 2003), suggesting a central role for these glia in nigro-striatal degeneration, irrespective of etiology. Microglia may be especially susceptible to mechanisms of aging. Their maintenance is proposed to be dependent on self-renewal rather than replenishment by peripheral blood precursors (Ajami *et al.*, 2011; Prinz and Priller, 2014), and their phagocytic function could diminish with age (Bliederhaeuser *et al.*, 2016), which could be highly significant in age dependent neurodegenerative conditions such as LBD. Systemic infections or disease, which rise in number with age, could also lead to priming of microglia, such that their response is exaggerated and damaging to nearby neurons leading to cognitive decline (Perry and Holmes, 2014). It has also been proposed that an initial stimulus that triggers microglial activation could persist in neurodegenerative disorders leading to repeated cyclical chronic neuroinflammation causing neuronal dysfunction and cell death (Gao and Hong, 2008; Tansey and Goldberg, 2010). The specificity of these changes to Lewy body dementias is unclear.

Astrocytes are the primary glial cells of the CNS, involved in brain homeostasis: supporting neurons and regulating the extracellular balance of fluid, ions and neurotransmitters. They also have an inflammatory response, with an ability to secrete cytokines and chemokines and activate the adaptive immune system. In comparison to microglia, astrocytes have been less well studied in neurodegeneration, however evidence is emerging of their potential as regulators of inflammation, in both protective and detrimental roles (Colombo and Farina, 2016).

5.4 Alpha Synuclein and Neuroinflammation

Alpha-synuclein is the main component of Lewy bodies (Spillantini *et al.*, 1997) which characterize LBDs pathologically, and the likely driving force behind the disease process, hence the interaction between this protein and microglia appears to be critical. Alpha-synuclein inclusions in neurons and glia are associated with DLB and PDD, as well as PD and multiple system atrophy. In DLB and PDD, the inclusions are neuronal and in the form of Lewy bodies (Spillantini *et al.*, 1997). Lewy neurites are also common in these

disorders, consisting of coarse dystrophic neurites immunoreactive for α-synuclein within affected neurons. With 140 amino acids, α-synuclein's possible intracellular forms include monomeric (Fauvet *et al.*, 2012; Lashuel *et al.*, 2013) or a relatively stable folded tetramer (Bartels, Choi and Selkoe, 2011; Wang *et al.*, 2011).

Many studies have found evidence of α-synuclein's ability to activate microglia and induce dopamine cell loss (Zhang *et al.*, 2005, 2007; Theodore *et al.*, 2008; Hoffmann *et al.*, 2016), including monomeric wild-type and mutant forms as well as extracellular oligomeric conformations and fibrils. Indeed, neuron-glia cultures depleted of microglia have been shown to be resistant to α-synuclein induced dopaminergic neurotoxicity (Zhang *et al.*, 2005). The initiation of the innate response occurs through pattern-recognition receptors (PRRs) expressed on CNS cells (for example the toll-like receptor (TLR)) through activation by pathogen associated molecular patterns or danger associated molecular patterns.

Recently the focus has been on possible mechanisms of interaction. Models of PD have been used to study this relationship rather than models of DLB, with overexpression of α-synuclein in the substantia nigra using viral vectors, the most common. A survey of the literature shows several potential mechanisms (see Table 5.1).

A number of immunomodulatory proteins and compounds are implicated in α-synuclein microglial recognition, chemotaxis, activation and response. TLRs 1 (Daniele *et al.*, 2015), 2 (Kim, Kågedal and Halliday, 2014; Daniele *et al.*, 2015) and 4 (Fellner *et al.*, 2013) are PRRs key to the innate response machinery and have been reported as having a role in recognition of α-synuclein by microglia. Microglia exposed to higher-ordered oligomers (but not monomers) of α-synuclein changed to an amoeboid, phagocytic morphology with increased secretion of tumor necrosis factor α (TNF-α) that was reduced by inhibition of the TLR 1/2 complex (Daniele *et al.*, 2015). A separate study found only β-sheet rich oligomeric conformations of α-synuclein could activate microglia via TLR 2, but both aggregated and non-aggregated forms could activate microglia through TLR 4. Furthermore pro-inflammatory cytokine/chemokine release was completely eliminated in TLR 2 knockout mouse microglia exposed to α-synuclein, but remained unaffected in TLR 4 knockout mouse microglia (Kim *et al.*, 2013). Selective activation of TLR 4 rather than TLR 2 receptors in transgenic α-synuclein mouse models also led to increased clearance of α-synuclein, improved motor performance and rescue of nigro-striatal neurons (Venezia *et al.*, 2017). In addition, human oligomeric α-synuclein injected into mouse hippocampi inhibited memory function, which was prevented by TLR

2 inhibition but not TLR 4 knockout (La Vitola *et al.*, 2018). This suggests recognition of oligomeric α-synuclein by TLR 2 leads to inflammation and dysfunction, whereas TLR 4 receptors respond with phagocytosis and cellular protection.

Another molecule which could feature in the initiation of microglia activation is fractalkine, a membrane bound chemokine which acts on its receptor (CX3CR1) on microglia to suppress production of inflammatory molecules. The soluble secreted form of fractalkine had a protective function in an animal model of α-synuclein overexpression, suggesting loss of this membrane bound chemokine could lead to neuronal loss through microglia mediated cell damage (Nash *et al.*, 2015). Deletion of CX3CR1 reduces microglial phagocytosis and MHC class II (MHCII) expression in response to α-synuclein, but does not increase neuronal loss (Thome, Standaert and Harms, 2015).

Alpha-synuclein, in extracellular aggregated form, has been shown to be a chemoattractant through CD11b receptors on microglia (S. Wang *et al.*, 2015). Also, the β1-integrin subunit, which forms transmembrane adhesion molecules has been reported as being required for the morphological changes and migration of microglia seen in the presence of extracellular α-synuclein (Kim *et al.*, 2014).

Once microglia are activated, interleukin-1 (IL-1) appears to be a key cytokine in promoting an inflammatory response. IL-1α and β knockout mice did not show loss of dopamine neurons or behavioral deficits seen in wild-type mice in a PD model, utilizing lipopolysaccharide (LPS) injections into the substantia nigra. LPS injections have been shown to produce microglial activation, cytokine release and subsequent dopaminergic cell loss in the substantia nigra (Sharma and Nehru, 2015). TNF-α knockout mice however showed similar results to wild-type mice (Tanaka *et al.*, 2013), indeed TNF-α may have a role in promoting α-synuclein accumulation (M.-X. Wang *et al.*, 2015). Galectin-3 has also been shown to be important for the inflammatory effect of α-synuclein. Its inhibition significantly reduced cytokine release by microglia in response to aggregated α-synuclein (Boza-Serrano *et al.*, 2014).

Leucine-rich repeat kinase 2 (LRRK2) is a protein expressed on microglia when they are in their inflammatory state and has been shown to have a significant role in α-synuclein mediated microglial activation and subsequent cell loss, with LRRK2 knockout mice being protected from α-synuclein overexpression (Daher *et al.*, 2014) as were mice treated with LRRK2 inhibitors (Daher *et al.*, 2015). LRRK2 knockout mice also exhibited increased clearance of α-synuclein compared to wild-type mice (Maekawa *et al.*, 2016).

Yet mutations in LRRK2 are associated with PD (see Genetics section), suggesting the contribution of this kinase to PD pathology is unclear.

Another protein involved is NRF2, a transcription factor for a number of cell protection proteins that appears to have a protective role in the interaction (Lastres-Becker *et al.*, 2012) - activation leads to protection from α-synuclein toxicity and inflammation (Lastres-Becker *et al.*, 2016).

Several studies suggest the adaptive immune response is engaged by microglia following their activation. Knockout mice without Fc gamma receptors (FcγR), which are found on microglia and involved in facilitating phagocytosis through binding of IgG, showed reduced pro-inflammatory signaling in the presence of aggregated α-synuclein. Suggesting the latter could be triggering inflammation and antibody mediated cell damage through FcγR (Cao, Standaert and Harms, 2012). However, one specific subtype FcγRIIB, in the presence of aggregated α-synuclein, inhibits microglial phagocytosis, suggesting an alternative means of microglial dysfunction through these receptors in synucleinopathies (Choi *et al.*, 2015).

A knockout of all four murine MHCII complex genes prevented α-synuclein induced dopaminergic cell loss in a mouse model, strongly suggesting that CD4 T lymphocytes are critical to α-synuclein cell damage. Microglia, as the only resident cells expressing MHCII in the CNS, would be candidates for their recruitment, although infiltrating antigen presenting cells such as macrophages (or their precursors, monocytes) may also be involved (Harms *et al.*, 2013)(Harms *et al.*, 2018). Furthermore, mice with microglia deficient in prostaglandin E2, which is thought to have a role in lymphocyte proliferation, have increased resistance to MPTP mediated pathology (Jin *et al.*, 2007). In addition, inhibiting the JAK/STAT pathway that is known to underlie many aspects of the immune response, suppresses microglial activation, T-cell infiltration in the substantia nigra and neurodegeneration in mouse models of α-synuclein over-expression (Qin *et al.*, 2016).

Inflammatory stimuli can also lead to truncation of α-synuclein, through activation of an inflammatory enzyme – caspase 1, and subsequent aggregation and neurotoxicity in neuronal cell cultures (Wang *et al.*, 2016). Such a pathway could lead to cell death independently or synergistically with microglial activation.

5.5 Imaging of Neuroinflammation and Neuronal Dysfunction

Imaging studies have shown an association between neuroinflammation *in vivo* and cognitive dysfunction. Microglial activation as a marker of neuroinflammation has been identified in PD and PDD (Fan *et al.*, 2015) (see Table 5.2), in the majority of cases using ^{11}C-*R*PK11195 (PK11195), a PET ligand that binds to a translocator protein (TSPO) found on microglia in their activated state. Extensive microglial activation has similarly been identified in another α-synucleinopathy: multiple systems atrophy (Gerhard *et al.*, 2003), as well as other degenerative conditions, including AD, a condition which shares some of the pathological features of LBD (Cagnin *et al.*, 2001; Edison *et al.*, 2008; Colom-Cadena *et al.*, 2013)

An association between microglial activation in the midbrain and dopaminergic loss in the dorsal putamen has been found in the early stages of PD (disease duration less than 2.5 years), both contralateral to the clinically affected side, with levels of activation correlating with severity of motor impairment measured by the Unified Parkinson's Disease Rating Scale (UPDRS) (Ouchi *et al.*, 2005). In the later stages of disease (disease duration range 0.5 - 21 years), there is extensive microglial activation, with the basal ganglia, cortex and pons all showing significantly increased levels. The substantia nigra was however spared. Follow-up scans in eight of these subjects (after 18-28 months) showed no significant change in microglial activation from baseline despite a clear deterioration in disability as measured using the UPDRS. Cognition was however not assessed longitudinally (Gerhard *et al.*, 2006). The authors also noted a clear overlap in the areas of microglial activation and the regions proposed by Braak et al. (Braak *et al.*, 2003) in their study of PD pathology. Another longitudinal study, this time with a second generation TSPO ligand [^{11}C]-DPA713, found increased microglial activation (compared to controls), spreading in cortical regions (temporal and occipital) in the same subjects over one year, despite no change in mini mental state examination (MMSE) scores (Terada *et al.*, 2016). Second generation ligands are reported to have a higher sensitivity to TSPO (Kobayashi *et al.*, 2018), however their affinity to TSPO depends on the expression of a polymorphism in the gene for this receptor unlike PK11195 (Owen *et al.*, 2012). Yet participants in this latter study were not assessed for genotype, calling into question the validity of the differences between patients and controls.

In PDD subjects, there is increased cortical microglial activation compared to control subjects, however levels of activation were also increased in comparison to PD cases, but just in the left parietal lobe (Edison *et al.*, 2013).

In DLB, increased microglial activation in the substantia nigra and putamen, plus several cortical regions was found in a pilot imaging study of six cases of less than one year's disease duration (Iannaccone *et al.*, 2013). That microglial activation occurs in more widespread regions in early DLB, where there is greater cognitive dysfunction compared to early PD, strengthens the link between microglial activation and cognitive function.

A relationship between microglial activation and cognitive function has been found in PDD, where cortical activation levels inversely correlated with MMSE in temporo-parietal, occipital, and frontal cortical regions (Edison *et al.*, 2013; Fan *et al.*, 2015). Fan et al. (Fan *et al.*, 2015) also demonstrated a significant negative correlation between whole brain levels of microglial activation and glucose metabolism. Within the temporo-parietal cortex there was voxel by voxel significant inverse correlation between levels of microglial activation and glucose metabolism in the immediate vicinity suggesting local damage, but the areas of correlation were small. Femminella et al. went further and demonstrated microglial activation within cortical and subcortical areas in PDD subjects correlated inversely with hippocampal volume and negatively with hippocampal glucose metabolism (Femminella *et al.*, 2016).

Small clusters of positive correlations were also found between PK11195 binding and amyloid load (as determined by [^{11}C]Pittsburgh compound B (PIB), a marker of fibrillary amyloid load) in PDD subjects, but only in the parietal lobe and anterior cingulate, as opposed to AD subjects in whom there was a stronger correlation between amyloid load and microglial activation. There was however little amyloid deposition found in PDD cases overall (Fan *et al.*, 2015). Proteins other than amyloid, such as α-synuclein or tau, could be triggering microglial activation in PDD, however currently there are no α-synuclein PET ligands available to demonstrate this and tau ligand imaging is in its early stages and as yet there are no studies investigation the relationship between tau and inflammation.

Overall small scale studies with *in vivo* imaging have suggested that in PD, PDD and in a small preliminary report of DLB, there is early microglial activation. But, this does not appear to increase over time in regions once it is established. Early microglial activation in synucleinopathies is further supported by PK11195 studies in patients with rapid eye movement (REM) sleep behaviour disorder - a condition now considered to be a prodromal stage of synucleinopathies(Högl, Stefani and Videnovic, 2018), which show increased binding in the substantia nigra (Stokholm *et al.*, 2017) and occipital cortex (Stokholm *et al.*, 2018), prior to any motor or cognitive impairment .

The evidence for extensive microglial activation in LBDs, in an immunologically privileged site such as the brain, is highly significant. Immune responses are tightly controlled and yet there is widespread inflammatory cell activation, starting early and present chronically during the disease.

5.6 Pathological Studies

Pathological studies further support a role for inflammation. Large numbers of microglia were reported to be HLA-DR-positive, which can indicate activation, in the substantia nigra of PD and PDD cases together with Lewy bodies in association with a reduction in dopaminergic cells. In the PDD cases HLA-DR positive microglia were also found in the hippocampus, though this was associated with neuritic plaques and tangles suggestive of AD pathology (McGeer *et al.*, 1988). Increased microglial expression of MHCII has also been reported in transenterohinal, cingulate and temporal cortices in PD (Imamura *et al.*, 2003).

In a post-mortem study of controls, idiopathic Lewy body disease patients and PD subjects, different patterns of inflammatory cytokine changes were found in the substantia nigra and striatum. Microglial HLA DR expression in the substantia nigra was found to be both intense and reduced in PD cases. In the striatum, tyrosine hydroxylase fibers were lower in PD compared to controls, but those which survived had particularly intense microglial HLA DR staining (Walker *et al.*, 2016).

The presence of CD4 (as well as CD8) T lymphocytes within the substantia nigra of PD cases at post-mortem has also been found (Brochard *et al.*, 2009). In addition, concentrations of interleukin-1β, interleukin-6 and transforming growth factor-α were higher in the striatal regions of post-mortem PD brains compared to controls (Mogi, Harada, Kondo, *et al.*, 1994). Complement proteins were also found with Lewy bodies within this region in PD (Loeffler, Camp and Conant, 2006). Furthermore TLR 2 expression is increased in PD brains and correlate with α-synuclein deposits. TLR 2 was found on neurons and microglia, the former correlating with disease duration (Dzamko *et al.*, 2017). Alpha-synuclein deposits have also been reported in the astrocytes of PD patients within the brainstem (Wakabayashi *et al.*, 2000) and cortex, adjacent to Lewy bodies and Lewy neurites (Braak, Sastre and Del Tredici, 2007).

In DLB cases, both complement proteins and MHCII positive microglia are associated with Lewy body containing degenerated neurons on autopsy, suggesting microglial

involvement (Togo *et al.*, 2001). An increase in MHCII positive microglia has also been reported, positively correlating with the number of Lewy bodies regionally (Mackenzie, 2000). However this was not as high as in those cases with concomitant senile plaques and a second study has shown a lack of MHCII positive microglia in the absence of neuritic plaques in DLB (Shepherd *et al.*, 2015). Indeed, Streit and Xue report that Iba1 staining which identifies all microglia, did not identify hypertrophic microglia suggestive of activation in DLB compared to controls in the frontal or temporal cortices. CD68 staining was raised in DLB cases, however this is a label for lipofucin deposits in microglial cells, that could indicate activated phagocytic microglia or senescent microglia that have accumulated lipofucin with age (Streit and Xue, 2016). Bachstetter and colleagues found that dystrophic microglia, rather than hypertrophic microglia were the predominant subtype in the hippocampus of DLB cases, suggesting hypofunction rather than a pro-inflammatory role (Bachstetter *et al.*, 2015).

In addition a recent study showed a correlation between changes in the anti-inflammatory marker CD200 or pro-inflammatory marker intercellular adhesion molecule-1 with amyloid plaques and tau tangles but not Lewy bodies in patients with DLB (Walker *et al.*, 2017). A comparison of middle aged healthy controls, rapidly progressive (less than two years between first symptom and death) DLB and other DLB cases, found no change in expression of a limited set of inflammatory genes between groups, but did find TNF-α protein levels were higher in the rapidly progressive group compared to controls (Garcia-Esparcia *et al.*, 2017).

Hence, there is some evidence of inflammation but so far there is an absence of a link between microglia and pathological protein deposition in both PDD and DLB. Pathological studies in DLB vary in their findings dependent on the marker used to identify microglia. Whilst there is no evidence of morphological change suggestive of activation, MHCII expression and possibly phagocytosis and dystrophic changes appear to be increased in patients with DLB. It should however be noted that autopsy studies are by definition at the end-stage of the disease process and may not be reflective of active disease mechanisms, especially those relevant at early stage of disease.

5.7 Genetic Studies

Genetic studies have identified polymorphisms in genes coding IL-1β, TNF-α and TREM2 as risk factors for PD. Up to a doubling of risk has been reported amongst

carriers of a genotype of IL-1β that is associated with increased gene expression (McGeer, Yasojima and McGeer, 2002; Wahner *et al.*, 2007). Those carrying the homozygous variant genotype TNF-α-308, a variant which is thought to be a stronger transcriptional activator, experience doubled risk (Wahner *et al.*, 2007). Overall the results from these two small studies are consistent with a gene dosing effect for these two powerful cytokines. A rare variant of the microglial receptor TREM2, that leads to loss of function, was found to be another risk factor for PD in a study of 1493 cases compared to 1957 controls (Rayaprolu *et al.*, 2013). Missense mutations in the LRRK2 gene are found in 1-2% of patients with PD, which codes for a kinase that is highly expressed in immune cells, and could play a role in the formation of the inflammasome - signaling complexes that play an important role in the inflammatory response (Alessi and Sammler, 2018).

Genome wide association studies (GWAS) provide further evidence for inflammatory pathology in PD. Polymorphisms in HLA regions that code segments of the MHCII molecule present increased risk. A strong association was found within non-coding intron 1 of HLA-DRA (in a study of 2000 cases and 1986 controls) by Hamza and colleagues (Hamza *et al.*, 2010), with subsequent large-scale meta-analyses of single nucleotide polymorphisms (SNP) confirming associations amid the HLA-DR locus, with both HLA-DRB5 (Nalls *et al.*, 2011) and HLA-DQB1 (Nalls *et al.*, 2014) identified. Wissemann and colleagues (Wissemann *et al.*, 2013) found loci that predisposed to, as well as protected from, PD within the same 2000 PD and 1986 control GWAS dataset initially analyzed by Hamza et al. (Hamza *et al.*, 2010), and replicated these in a further 843 cases and 856 controls. The strongest association was again intron 1 of the HLA-DRA region, which regulates gene expression and linked to increased risk. This suggests HLA expression levels may play a key role in determining risk for PD. Indeed subjects homozygous for the G allele in this SNP, were found to have significantly increased MHCII expression, compared to subjects who did not have a single G allele. In addition, exposure to a common insecticide, pyrethroid, when combined with possession of the GG allele, significantly increased PD risk (Kannarkat *et al.*, 2015), suggesting a combination of environmental triggers and inflammatory processes may play a part in PD pathology.

Polymorphisms in genes associated with inflammation are yet to be identified as risk factors for PDD specifically. However in a GWAS study of 788 pathologically confirmed DLB cases and 2624 controls, ApoE, which may be involved in immune signaling, was identified as increasing risk (Guerreiro, Owen A. Ross, *et al.*, 2018). This was further

confirmed in a larger study of 1743 DLB (1324 pathologically confirmed) and 4454 controls (Guerreiro, Owen A Ross, *et al.*, 2018).

5.8 Blood Biomarkers

Elevated peripheral inflammatory markers both before and after the onset of PD, suggest inflammation is concurrent with the disease. Increased plasma interleukin-6 (IL-6), measured on average 4.3 years before diagnosis, is associated with increased risk of developing PD, with higher levels associated with higher risk (Chen *et al.*, 2008). After disease onset, levels of IL-6 (Dobbs *et al.*, 1999; Hu *et al.*, 2015), IL-1β (Hu *et al.*, 2015) and TNF-α (Dobbs *et al.*, 1999) are elevated compared to controls in PD, as are RANTES (regulated on activation, normal T cell expressed and secreted), a chemokine which attracts T-cells and high sensitivity CRP. RANTES levels also correlated with motor symptom severity (Rentzos *et al.*, 2007) and CRP with subsequent progression of motor impairment (Umemura *et al.*, 2015).

A change in peripheral blood lymphocyte subsets further suggests a role for the adaptive immune system. A decrease in the overall level of T-helper CD4 cells but a rise in the subset of activated T-helper cells is reported in PD cases compared to controls (Bas *et al.*, 2001).

In PDD, high sensitivity CRP is increased compared to controls, but a significant elevation was not found in PDD compared to PD (Song *et al.*, 2013). In DLB, one study has assessed inflammatory blood biomarkers in DLB and prodromal DLB, the latter defined as the presence of two core or suggestive features of DLB, in the absence of dementia. Whilst no changes were found in established disease, interleukin-10, interleukin-1β, interleukin-4 and interleukin-2 were higher in prodromal DLB than in controls (King *et al.*, 2017)

5.9 Cerebrospinal Fluid Biomarkers

Attempts to identify a reliable cerebrospinal fluid (CSF) biomarker for PD or PDD have so far been inconsistent. The main candidates include total α-synuclein, Aβ42, and β-Glucocerebrosidase (Parnetti *et al.*, 2013). Inflammatory cytokines TNF-α (Mogi, Harada, Riederer, *et al.*, 1994; Delgado-Alvarado *et al.*, 2017), IL-6 (Blum-Degen *et al.*, 1995; Müller *et al.*, 1998) and IL-1β (Blum-Degen *et al.*, 1995; Hu *et al.*, 2015) have also

been investigated with raised levels seen in the CSF of PD cases compared to controls. IL-1β levels in the CSF were associated with raised α-synuclein oligomers also in the CSF, suggesting a direct link with protein deposition (Hu *et al.*, 2015).

In a study of 22 cases of PD, IL-6 was found to associate inversely with disease severity as assessed by the UPDRS (Müller *et al.*, 1998). In a larger study of 62 cases, IL-6 was elevated in cases of PD with cognitive impairment compared to those without, the levels being negatively correlated to cognitive function. TNF-α and Interferon γ levels were however reduced in those with cognitive impairment in PD compared to control subjects (Yu *et al.*, 2014). A rise in the fractalkine:Aβ42 ratio in CSF is also associated with motor severity of PD (again measured by UPDRS) but not with disease duration (Shi *et al.*, 2011). An increase in this ratio could suggest increased inflammatory signaling and microglial activation. An increase in Leucine rich α2-glycoprotein (LRG), thought to be a marker of inflammation, is reported in the CSF and post-mortem tissue of PDD and DLB cases, compared to controls (Miyajima *et al.*, 2013).

In a longitudinal study of PD cases, the inflammatory protein YKL-40 was found to rise over 2 years in the CSF (Hall *et al.*, 2016). However when compared to AD cases, DLB and PDD subjects have lower levels of YKL-40 in their CSF (Wennström *et al.*, 2015; Janelidze *et al.*, 2016).

The focus in DLB however has been on the variations of Aβ peptides and tau as well as α-synuclein; a combination of biomarkers may be the best route to increase specificity and sensitivity (Mollenhauer and Trenkwalder, 2009; Schade and Mollenhauer, 2014). The inflammatory marker Procalcitonin has been found to be significantly raised in dementia subjects within the CSF, compared to controls, with the highest median level found in DLB cases (Ernst *et al.*, 2007).

5.10 Epidemiological Studies

There is limited support for neuroinflammation in PD from epidemiology studies. A meta-analysis of the association of NSAIDs and the risk of developing PD, showed a 15% reduction in incidence among users of non-aspirin NSAIDS, with analysis of ibuprofen alone showing a stronger protective effect. This effect was more pronounced among regular users (Gagne and Power, 2010). Whether PDD incidence was lower in those who developed PD despite taking NSAIDS was not considered.

A further meta-analysis showed conflicting results with no overall protective effect, however there were methodological differences including the inclusion of aspirin and studies where NSAID exposure was entirely within a 1 year of the diagnosis of PD. Nevertheless a slight protective effect for ibuprofen in lowering the risk of PD was still confirmed (Samii *et al.*, 2009). The evidence from these studies is however difficult to interpret because of variations in the drugs investigated, the duration of the drug treatment and the timing of administration in relation to disease onset.

Whether NSAIDs could reduce the risk of developing DLB has not yet been established.

5.11 A Role for the Adaptive Immune System

Despite the evidence of microglial activation and an interaction between α-synuclein and microglia, the precise mechanism and whether it is always detrimental to neurons remains unclear. A paucity of the relationship between Lewy bodies and antigen presenting activated microglia in post mortem studies was reported by Imamura et al. (Imamura *et al.*, 2003), indeed there was only a 20% association. This would suggest that Lewy bodies alone are not sufficient in themselves to trigger antigen presentation by microglia. In addition, increasing neuronal loss in the substantia nigra with lengthening disease duration was not associated with an increase in microglial activation, which is also reflected by *in vivo* PET studies (see above), implying a steady rather than escalating inflammatory response (Orr *et al.*, 2005),

Orr and colleagues (Orr *et al.*, 2005) also demonstrated that substantia nigra neurons were immunopositive for IgG in PD, whereas control cases' substantia nigra neurons as well as the visual cortex of PD cases showed negative immunoreactivity. Neuronal IgG labelling related to the degree of neuronal loss and microglial activation, with the authors suggesting humoral immune system involvement in the selective destruction of substantia nigra neurons.

Given that the MHCII complex has also been shown to be key in dopamine neuronal cell loss in mouse models (Harms *et al.*, 2013), it may be that an adaptive immune response is the final path to neuronal loss, following a switch in microglia function from protective to deleterious. Consistent with this theory is the genetic risk associated with HLA class II gene variation previously described, as well as the alteration in peripheral lymphocyte subsets found in PD cases (Bas *et al.*, 2001), and the evidence that T lymphocyte infiltration of the substantia nigra is found at post mortem in PD subjects (Brochard *et al.*,

2009) and in a mouse model of α-synuclein overexpression (Theodore *et al.*, 2008). In addition α-synuclein fibrils lead to striatal degeneration and invasion of MHCII positive monocytes in mouse models (Harms *et al.*, 2017) and increased microglial MHCII expression has been repeatedly found at post-mortem in Lewy body disorders (Mackenzie, 2000; Shepherd *et al.*, 2015).

It is possible initial protein clearance by microglia could be switched to a more harmful toxic function involving recruitment of the adaptive response ultimately leading to neuronal degeneration. For example due to peripheral inflammation or increased vulnerability of microglia through ageing, the latter supported by the identification of increased dystrophic microglia in DLB (Bachstetter *et al.*, 2015). The timing of treatment initiation would be key in such circumstances.

5.12 Conclusion

Evidence for the role of neuroinflammation in LBDs continues to accumulate, building on the evidence of neuroinflammation in AD and PD. Imaging studies lead the way in supporting neuroinflammation as a key part of the pathogen process in LBDs, supported by pathological and biomarker evidence. Future studies are required to further establish the presence of inflammation in DLB including imaging and peripheral biomarker studies.

Involvement of microglia in LBDs is signified by the presence of activation years before neuronal death as revealed by *in vivo* imaging. Microglial involvement is also supported by evidence of the activation of microglia by α-synuclein. Levels of activation however appear to remain relatively stable, which could indicate initiation and propagation of the disease process by microglia or alternatively a protective function that is eventually overcome (see Figure 5.1). In order to understand how inflammation affects disease progression in Lewy body dementia, studies need to try and link the nature and extent of microglial activation with peripheral markers and important indicators of disease severity such as protein deposition and the onset and progression of key cognitive and non-cognitive symptoms through longitudinal studies in established disease and in those at risk. A better understanding of these mechanisms and the stage within the disease at which they operate, could potentially lead the way to trials of novel immunomodulatory therapies.

Table 5.1: Potential mechanisms of interaction between α-synuclein and microglia

INTERACTION/ RECEPTOR	PROPOSED MECHANISM OF MICROGLIAL INTERACTION WITH α-SYNUCLEIN	PD MODEL	REFERENCES
TLR 1&2 complex	**Oligomeric α-synuclein induces a pro-inflammatory microglial phenotype through TLR 1/2 complex:** microglia exposed to oligomers of α-synuclein changed to an amoeboid, phagocytic shape, with increased secretion of TNF-α and interleukin-1b. TNF-α secretion was reduced by the addition of a TLR-1/2 complex inhibitor or by a MyD88 inhibitor.	Primary microglia cultures derived from mouse cortices were exposed to high-order oligomeric forms of purified human wild-type α-synuclein	(Daniele *et al.*, 2015)
Fractalkine (FKN) receptor	**Fractalkine receptor required for alpha synuclein phagocytosis and inflammatory response**: MHCII expression and IgG deposition in response to alpha-synuclein overexpression is attenuated by deletion of FKN.	Mouse model using overexpression of human α-synuclein via viral vector in wild-type and FKN knockout mice	(Thome, Standaert and Harms, 2015)
Secreted fractalkine receptor (sFKN)	**Secreted form of fractalkine is neuro-protective**: Soluble sFKN prevents reduction in tyrosine hydroxylase cell staining compared to controls and membrane bound fractalkine models when exposed to overexpression of α-synuclein, despite increased MHCII expression on microglia	Overexpression of human α-synuclein via viral vector combined with a variety of viral constructs of fractalkine	(Nash *et al.*, 2015)
CD11b receptor	**Alpha-synuclein binds to CD11b on microglia to direct microglial migration:** neuronal α-synuclein overexpression led to microglial migration toward neurons, which was reduced by antibodies to the CD11b receptor and diminished in CD11b knockout mice	Overexpression of human α-synuclein via viral vector in rat primary neuron-enriched cultures	(S. Wang *et al.*, 2015)
Galectin-3 (carbohydrate-binding protein and inflammatory mediator)	**Galectin 3 mediates microglial cytokine release**: Release of Interleukin-2 and Interleukin-12 after exposure to monomeric and aggregated forms of recombinant α-synuclein reduced by genetic down regulation or pharmacological inhibition of galectin-3	Microglia from wild-type and galectin-3 knockout mice	(Boza-Serrano *et al.*, 2014)

Leucine-rich repeat kinase 2 (LRRK2)	**LRRK2 required for microglial activation and dopaminergic degeneration**: Rats lacking LRRK2 demonstrated a significant reduction in microglial activation compared to wild type mice rats, when exposed to lipopolysaccharide (LPS) and were protected from dopaminergic neurodegeneration from α-synuclein overexpression.	Rats exposed to intracranial LPS injection or overexpression of human α-synuclein via viral vector	(Daher *et al.*, 2014)
β1-integrin	**Migration of microglia to disease affected regions is via β1-integrin**: β1-integrin inhibition reduced microglial morphological changes and motility (as shown by reduced wound healing)	Rat primary microglia exposed to α-synuclein conditioned medium (αSCM)	(Kim *et al.*, 2014)
Interleukin-1 (IL-1)	**IL-1 is required for microglial activation**: behavioral deficiencies that occurred in wild-type mice, following LPS administration did not occur in IL-1 knockout mice. Tyrosine Hydroxylase gene expression was similarly preserved in in IL-1 knockout but not wild-type mice.	Mouse model using intracranial LPS injection into wild-type and IL-1 (α and β) knockout mice	(Tanaka *et al.*, 2013)
MHCII Complex	**MHCII complex mediates microglial activation and dopaminergic cell loss**: overexpression of synuclein leads to induction of MHCII expression on microglia and genetic knockout of MHCII prevents microglial activation, IgG deposition and dopaminergic cell loss *in vivo*	Mouse model using overexpression of human α-synuclein via viral vector in wild-type and MHCII knockout mice	(Harms *et al.*, 2013)
TLR 4	**TLR 4 mediates microglial phagocytic activity and cytokine release in the presence of α-synuclein:** Microglial phagocytic activity was significantly reduced in TLR4 knockout microglia mice after treatment with different forms of α-synuclein; knockout mice also showed significantly reduced TNF- α production following treatment with α-synuclein.	Mouse primary microglia from wild type and TLR4 knockout mice challenged with cloned human α-synuclein from spinal cord cDNA	(Fellner *et al.*, 2013)
TLR 2	**TLR 2 mediates microglial activation by oligomeric α-synuclein**: TLR2 knockout mice exhibited significantly lowered microglial activation compared with wild type mice when exposed to α-synuclein overexpression; cytokine/chemokine gene induction following exposure to αSCM, was prevented by antagonizing TLR2 and by depletion	Mouse model using overexpression of human α-synuclein via viral vector in wild-type and TLR 2 knockout mice; oligomeric human α-synuclein proteins released from dSY5Y cells	(Kim, Kågedal and Halliday, 2014)

	of the TLR2 gene; and TLR2 was only activated by oligomeric alpha synuclein not the dimer or monomer forms.		
Fc gamma receptors (FcγR)	**FcγR mediates α-synuclein intracellular localization to autophagosomes and NF-κB pro-inflammatory signaling**: microglia internalized α-synuclein in a dense aggregated form in wild-type mice but a diffuse manner in FcγR knockout mice; FcγR knockout mice treated with α-synuclein also failed to trigger the enhancement of nuclear NF-κB p65 seen when wild-type mice are exposed to α-synuclein.	Primary microglial cultures from wild-type and FcγR knockout mice, treated with human α-synuclein	(Cao, Standaert and Harms, 2012)
NRF2 (NF-E2-related factor 2), a transcription factor	**NRF2 protects against α-synuclein mediated microglial activation and dopaminergic cell loss:** NRF2 knockout mice showed increased microglial activation and greater nigral dopaminergic neuronal loss than wild-type mice when exposed to α-synuclein overexpression; NRF2 knockout neurons were characterized by thick dendrites loaded with α-synuclein, similar in appearance to Lewy neurites and this was associated with reduced levels of the beta subunit (PSMB7) of the catalytic core 20S proteasome compared to wild-type mice	Mouse model using overexpression of human α-synuclein via viral vector in wild-type and NRF2 knockout mice	(Lastres-Becker *et al.*, 2012)
Prostaglandin E2 receptor subtype 2 (PGE2)	**PGE2 is key to regulation of aggregated α-synuclein levels:** microglia isolated from PGE2 knockout mice exhibited enhanced clearance of aggregated α-synuclein and showed increased resistance to MPTP with less aggregated α-synuclein in the substantia nigra and striatum.	Aggregated α-synuclein from human DLB cases incubated with wild-type and PGE2 knockout mice microglia	(Jin *et al.*, 2007)

Table 5.2: Evidence of *in vivo* microglial activation in PD, PDD and DLB from TSPO PET imaging studies

STUDY	PARTICIPANT NUMBERS (controls)	PARTICIPANT AGE (years)	PARTICIPANT MMSE	DISEASE DURATION (years)	REGIONS WITH INCREASED MICROGLIAL ACTIVATION COMPARED TO CONTROLS
(Ouchi *et al.*, 2005)	10 PD (10 controls)	Range: 43-72; Mean: 59.6	Range: 26-30; Mean: 28.3	Range: 0.4-2.5; Mean: 1.4	Midbrain contralateral to the clinically affected side
(Iannaccone *et al.*, 2013)	6 PD (11 controls)	Range: 60-74 ; Mean: 70.2	Range: 27-30; Mean: 29	Range: 0.6-1; Mean: 0.8	Putamen, substantia nigra
(Gerhard *et al.*, 2006)	18 PD (11 controls)	Range: 50-69; Mean: 59.2	Not specifically stated, screening tests normal in PD group	Range: 0.5-21; Mean: 8.6	Striatum, pallidum, thalamus, cortex (precentral gyrus, frontal lobe, anterior cingulate gyrus, posterior cingulate gyrus) and pons
(Edison *et al.*, 2013)	8 PD (10 controls)	Range: 58-75; Mean: 68.2	Range: 27-30; Mean: 28.8	Mean: 9.2	Cortex (temporal, parietal, and occipital regions)
(Terada *et al.*, 2016)	11 PD (12 controls)	Range: 62-80; Mean: 68.1	Range: 24-30; Mean: 26.3	Range: 1-8; Mean 3.1	*Cortex (initial scan: left fusiform and precentral gyrus; one year follow-up scan: left middle frontal, left precuneus, left inferior temporal, left parahippocampus, right inferior occipital, right postcentral gyrus, right superior parietal gyrus).
(Femminella *et al.*, 2016)	9 PDD (8 controls)	Range: 64-79; Mean: 69.3	Range: 18-25; Mean: 21.3	Not stated	Striatum, cortex (anterior cingulate gyrus, posterior cingulate gyrus, frontal lobe, temporal lobe, parietal lobe, occipital lobe)
(Fan *et al.*, 2015)	11 PDD (8 controls)	Range: 55-75; Mean: 68.4	Range: not stated; Mean: 22.1	Not stated	Anterior cingulate gyrus, posterior cingulate gyrus, frontal lobe, temporal lobe, parietal lobe, occipital lobe, medial temporal lobe, amygdala and hippocampus
(Edison *et al.*, 2013)	11 PDD (10 controls)	Range: 56-80; Mean: 69.3	Range: 16-26; Mean: 21.8	PD duration mean: 10.6; Dementia duration mean: 3.5	Striatum, cortex (frontal, temporal, parietal, anterior and posterior cingulate gyrus, and occipital cortical regions)
(Iannaccone *et al.*, 2013)	6 DLB (11 controls)	Range: 62-82 ; Mean: 72	Range: 19-30; Mean: 24	Range: 0.7-1; Mean: 0.8	Caudate, putamen, thalamus, substantia nigra, cortex (frontal lateral, parietal lateral, temporal lateral, temporal pole, precuneus, occipital medial, occipital lateral, anterior cingulate, posterior cingulate) and cerebellum

All studies used ^{11}C-PK11195 other than Terada et al which used ^{11}C-DPA713. * Note no assessment was made of participants and controls as to whether they were low, mixed or high affinity binders, despite the use of ^{11}C-DPA713 which has variable affinity to TSPO dependent on a person's genotype. ^{11}C-PK11195 binding is insensitive to genotype (Kobayashi *et al.*, 2018).

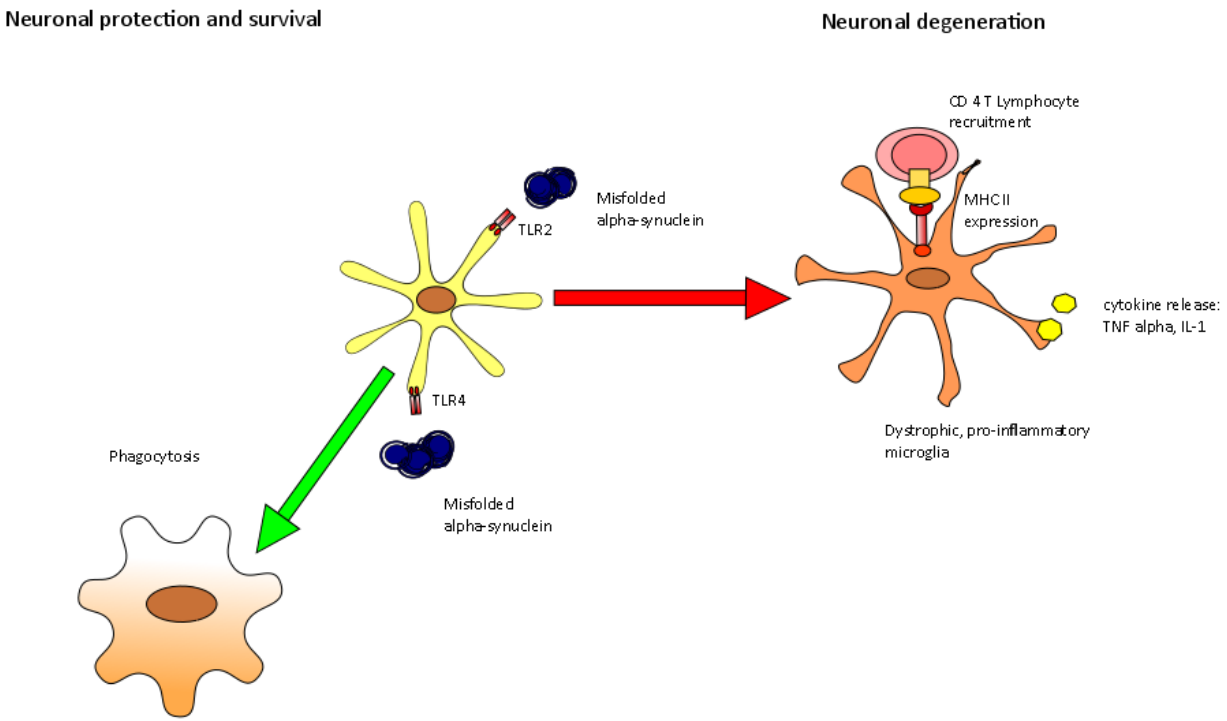

Figure 5.1 Possible mechanisms of microglial activation. Studies of mouse models suggest misfolded alpha-synuclein can activate microglia through a number of pathways. TLR2 and TLR4 may trigger different microglial phenotypes that result in detrimental or beneficial effects respectively, on the surrounding cells.

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Chapter 6

Inflammation in dementia with Lewy bodies – Introduction and Methods

6.1 Introduction

Neuroinflammation is increasingly considered as a contributor to dementia pathogenesis (Amor *et al.*, 2014), and a potential target for novel disease-modifying therapeutic strategies. The previous chapter reviewed the literature for evidence of inflammation in Lewy body dementia (LBD) and Parkinson's disease (PD), a condition with which it shares common pathology. There is evidence that α-synuclein aggregates are able to interact with a range of components of the immune system including microglia, potentially providing the substrate for an inflammatory response (Zhang *et al.*, 2005, 2007; Theodore *et al.*, 2008; Hoffmann *et al.*, 2016). In PD, inflammation has been identified with a range of methodologies, including pathological, genetic, epidemiological and cytokine assessment (Dobbs *et al.*, 1999; Imamura *et al.*, 2003; Wahner *et al.*, 2007; Brochard *et al.*, 2009; Hamza *et al.*, 2010). Similar evidence has also been identified in Alzheimer's disease (AD) (Lee *et al.*, 2010; McGeer and McGeer, 2013; Morales *et al.*, 2014; Latta, Brothers and Wilcock, 2015; Lai *et al.*, 2017; Passamonti *et al.*, 2018).

Recent studies suggest inflammation may occur early. Inflammation is reported in the early stages of Alzheimer's disease as well as in mild cognitive impairment (MCI) even before the onset of dementia (Okello *et al.*, 2009; Hamelin *et al.*, 2016), though there is a need for further evidence as some studies of patients with MCI report no differences in inflammation relative to controls (Wiley *et al.*, 2009; Kreisl *et al.*, 2013; Schuitemaker *et al.*, 2013).

In Parkinson's disease, PET imaging shows early inflammation *in vivo* in the brain stem before extending cortically as the disease progresses. By the onset of dementia, increased microglial activation appears to be widespread (Ouchi *et al.*, 2005; Gerhard *et al.*, 2006; Fan *et al.*, 2015). Persons affected by rapid eye movement (REM) sleep behaviour disorder, that is now recognised as a prodromal stage of synucleinopathies (Högl, Stefani and Videnovic, 2018), show elevated microglial activation in the substantia nigra (Stokholm *et al.*, 2017).

In addition, direct evidence of inflammation in Lewy body disease is growing, with MHC class II positive activated microglia closely associated with Lewy body positive neurons at post-mortem (Mackenzie, 2000)(Togo *et al.*, 2001), though not all studies report inflammation (Streit and Xue, 2016), meaning further pathological evidence is needed. Activated microglia were also identified *in vivo* on PET in one small case series of dementia with Lewy body (DLB) subjects (Iannaccone *et al.*, 2013). Exploratory next generation gene sequencing indicates an inflammatory component in DLB pathology,

with specific antigen presentation alleles (HLA-DPA1/DPB1) increasing risk (Peuralinna *et al.*, 2015). Elevated interleukins have also been reported in prodromal DLB, although not the established disease (King *et al.*, 2017). In Parkinson's disease dementia (PDD), another form of dementia associated with Lewy bodies, a rise in c-reactive protein has been identified, after the onset of dementia (Song *et al.*, 2013).

Inflammation represents a potential means of modifying disease progression in dementia. Whether central or peripheral, therapies attenuating inflammation may be able to slow or even halt progressive neurodegeneration. Anti-inflammatory treatments already exist (Martin *et al.*, 2016), meaning that in contrast to the disappointing results thus far for the discovery of therapies targeting protein accumulation in dementia (Mo *et al.*, 2017), therapies targeting inflammation could be brought into clinical practice more quickly. The identification of inflammation as an early part of the disease process would increase its usefulness as a target with treatment then possible in the prodromal phase.

However, more definitive evidence of peripheral and central inflammation *in vivo* in DLB is needed, as well as further evidence of the stage(s) in the disease process at which inflammation occurs, essential information to plan future therapeutic studies. A deeper understanding of the *in vivo* relationship between central and peripheral inflammation in the same patients is also required, to better elicit the role of inflammation in the pathophysiology of the disease and its effect on the clinical syndrome.

To assess for these differences, this study undertook PET imaging with ^{11}C-PK11195 (PK11195), a marker of microglial activation *in vivo* within the brain, and tested for peripheral inflammatory cytokines in patients with DLB and healthy controls.

If consistent with the studies in the closely related conditions of PD and AD, patients with DLB would have increased central and peripheral inflammatory changes when compared to controls. These changes would also likely vary according to disease severity, with more pronounced changes early in disease as has been found in Parkinson's disease and, in some studies, Alzheimer's disease. Accordingly, cognitive and motor performance in each subject was assessed for comparison with any central or peripheral inflammatory changes.

Finally, in view of the concurrent beta-amyloid pathology found in many DLB patients (Colom-Cadena *et al.*, 2013), concomitant amyloid protein deposition was also tested using ^{11}C-Pittsburgh compound B (PiB) PET to assess whether amyloid load correlated with inflammation centrally or peripherally.

6.1.1 Hypotheses

This study tested the following hypotheses - that:

1) there is an increase in brain microglial activation in DLB as measured by PK11195, compared to similarly aged healthy controls, that varies according to disease severity, with more pronounced changes seen early in disease,

2) there is an increase in peripheral inflammation in DLB compared to similarly aged healthy controls as seen in AD and PD, as measured by blood inflammatory marker levels,

3) levels of microglial activation correlate with:

    a) key clinical symptoms in cognition and motor function,

    b) peripheral markers of neuroinflammation,

    c) the cortical amyloid load as assessed with PiB PET, and

4) the differences in microglial activation between DLB and controls would be in regions predicted by known anatomical correlates of the clinical syndrome.

6.1.2 Sample size

The present study aimed to recruit DLB subjects with a range of disease durations and similarly aged controls to investigate PK11195 binding in relation to disease severity. 16 DLB patients and controls would provide a power of 80% power to detect a standardised effect size of 1.0 between the groups, with an alpha of 0.05 (Hulley *et al.*, 2007):

$N = 2 (z_\alpha + z_\beta)^2 / (\delta/\sigma)^2$; where $\alpha=0.05$, $\beta=0.20$, $\delta/d = 1.0$

$N = 2(1.96 + 0.84)^2/(1.0)^2$

N = 15.68 or 16 rounded up.

The preliminary data from the small case series by Iannaccone *et al.* (Iannaccone *et al.*, 2013) showed that the mean PK11195 binding potential in the caudate was 0.08 for controls, and 0.19 for DLB subjects and that the standard deviation of the binding

potential in controls was 0.03. Hence the level in the DLB subjects was more than one standard deviation above the mean in the controls. Similar values were reported in the majority of the other regions tested, including the precuneus, putamen, occipital lateral cortex and occipital medial cortex. However the study by Iannaccone did not have similarly aged controls (no mean age was given - only a range of 29 to 60 years, compared to a mean of 72 years for the DLB group) which could explain the low mean binding potential and standard deviation in the control group. It also only studied early (of less than one year disease duration) DLB subjects, for whom PK11195 binding is also expected to be high as hypothesised. The mean levels of binding and standard deviation in controls could be higher in older control patients and also closer to DLB patients with severe disease; hence a standardised effect size of 1.0 has been used for this study.

This study aimed to recruit 16 controls and between 16 and 20 DLB subjects, allowing for any attrition in patients between scans and any loss of quality in the scan data (due to patient movement for example).

6.2 Methodology

6.2.1 Participants

All participants were aged over 50 years and had sufficient proficiency in English for cognitive testing. Nineteen patients with “probable” DLB as defined by both 2005 and 2017 consensus criteria (McKeith *et al.*, 2005, 2017), and 26 healthy controls with similar ages and gender were recruited. Exclusion criteria were (1) acute infection, (2) a contra-indication to MRI, or a history of any of the following: (3) major psychiatric disorder (e.g. major depression), (4) neurological disorder (except a diagnosis of DLB in DLB subjects), (5) head injury, or (6) systemic inflammatory disorder (e.g. systemic lupus erythematosus, rheumatoid arthritis or Crohn’s disease).

Patients were identified from the specialist memory clinic at the Cambridge University Hospitals NHS Trust (CUH), other local memory clinics, from the Dementias and Neurodegenerative Diseases Research Network (DeNDRoN) volunteer registers or the Join Dementia Research platform (https://www.joindementiaresearch.nihr.ac.uk). Healthy controls were recruited via DeNDRoN or Join Dementia Research as well as from spouses and partners of patient participants.

6.2.2 Clinical Assessments

All participants underwent an initial assessment that included neuropsychological and cognitive testing (including Mini Mental State Examination (MMSE) and Addenbrooke's Cognitive Examination-Revised (ACE-R)), severity of parkinsonism (Unified Parkinson's Disease Rating Scale part III - motor (UPDRS)) and demographic measures.

6.2.3 MRI and PET Imaging

All participants underwent MRI on a 3 Tesla Siemens Magnetom Tim Trio, Verio or Skyra scanner (www.medical.siemens.com). Each MPRAGE (magnetization-prepared rapid acquisition gradient-echo) T1-weighted sequence was non-rigidly registered to the ICBM2009a template brain using ANTS (http://www.picsl.upenn.edu/ANTS/) and the inverse transform was applied to the modified Hammers atlas (resliced from MNI152 to ICBM2009a space) to bring the regions of interest (ROI)s to subject MRI space, to which the PET data described below was co-registered. The T1 scanning protocol was as follows: 176 slices of 1.0 mm thickness, TE= 2.98 ms, TR = 2300 ms, flip angle =9°, acquisition matrix 256x240; voxel size = 1x1x1 mm^3.

19 DLB and 16 control group participants underwent PK11195 PET imaging to assess the extent and distribution of microglial activation, using a GE Advance PET scanner (GE Healthcare, Waukesha, WI) or a GE Discovery 690 PET/CT, with attenuation correction provided by a transmission scan or a low dose CT scan, respectively. The emission protocol for PK11195 were 75 minutes of dynamic imaging consisting of 55 frames starting concurrently with a 500 MBq PK11195 injection. Binding in each ROI was quantified using non-displaceable binding potential (BP_{ND}) determined with the simplified reference tissue model previously validated for PK11195 (Turkheimer *et al.*, 2007) and corrected for cerebrospinal fluid (CSF) partial volumes. 16 of the DLB participants also underwent PiB imaging to determine cortical amyloid burden, with 550 MBq of PiB injected as a bolus and imaging performed for 30 minutes starting at 40 minutes post-injection. PiB data were quantified using standardized uptake value ratio (SUVR) by dividing the mean CSF-corrected radioactivity concentration in each Hammers atlas ROI by the corresponding mean CSF-corrected radioactivity concentration in the reference tissue ROI. Participants were considered amyloid positive if the average SUVR value across the cortical ROIs was > 1.5 (Hatashita and Yamasaki, 2010). The radiotracers were produced at the Wolfson Brain Imaging Centre (WBIC) Radiopharmaceutical

Chemistry laboratories. Both of the ^{11}C-labelled compounds were produced using the GE PETtrace cyclotron, a 16MeV proton and 8MeV deuteron accelerator. PK11195 was prepared using the "Disposable" synthesis system or GE TRACER lab FX-C module, whereas PiB was prepared using the GE TRACER lab FX-C module.

6.2.4 Cytokine Assessments

Blood samples were obtained from all participants, allowed to clot for at least 30 minutes, centrifuged to isolate serum, then aliquoted and stored at -70 degrees until further analysis as below.

Assays were carried out by the Core Biochemical Assay Laboratory, Cambridge University Hospital using the MesoScale Discovery V-Plex Human Cytokine 36 plex panel and five additional cytokine assays: high sensitivity c-reactive protein (using Siemens Dimension EXL autoanalyser), tumor necrosis factor receptor 1 (using the electrochemiluminescence immunoassay from MesoScale Discovery) and interleukin-34 (IL-34), YKL-40 (Chitinase-3-like protein 1), plus macrophage colony stimulating factor 1 (all using BioTechne R&D Systems kit,). Dilutions were made in accordance with manufacturer recommendations. Each assay was performed in duplicate, with the mean taken for the purposes of analysis.

6.2.5 Statistical Analysis

Statistical analysis was completed using IBM SPSS Statistics software (version 25) and the support vector machine (SVM) analysis carried out with R: R Foundation for Statistical Computing, Vienna, Austria (URL https://www.R-project.org/).

Demographics were compared using student's t-test for continuous variables and chi-squared test or Fisher's exact test for categorical variables. To compare cytokine levels between the DLB and cytokine control group, a repeated measures general linear model tested for the effect of group and group cytokine interaction, with age and gender included as covariates of no interest. The majority of the cytokine assay results were positively skewed, hence all cytokine measurements were transformed with log10 (x +1) prior to analysis to improve normality for the general linear model. PK11195 binding between the DLB and PET control group was also compared using a repeated measures general linear model, with age, gender and education included as covariates of no interest.

To study further whether cytokine profiles or PK11195 binding in regions of interest could differentiate subjects according to group, a support vector machine was used, with feature selection to select the best variables from these datasets and identify the highest rate of accuracies that could be obtained for classification into groups. The SVM model was trained with leave one out cross-validation and a linear kernel tuned to provide the optimum balance between a wide margin between support vectors in the hyperplane and a small number of misclassified data points. Application of the SVM across different training group partition sizes, where each subject was randomly allocated to testing or training identified: (i) the training and testing split that provided the highest accuracies, and (ii) an order of influence of each variable as support vectors. Next feature selection was carried out, similar to that previously used to identify optimum blood biomarker panels in Alzheimer's disease (Long *et al.*, 2016). Features were individually added in order of increasing influence to create an enlarging panel of variables. For each panel, the SVM was repeated 5000 times, each with randomly allocated training groups from the full list of sample subjects, to obtain the mean accuracy for classification into groups. Once the panel with the highest accuracy was obtained, features were further selected within this subset based on changes in accuracy following their removal from the panel, to identify a set of features that recorded the peak accuracy.

Correlations between clinical factors (disease duration and disease severity measured through ACE-R, and UPDRS), regional PK11195 BP_{ND}, amyloid SUVR, and cytokine

levels, were assessed with Pearson partial correlation, with age, gender and education as co-variates of no interest. To correct for multiple comparisons, the Benjamini-Hochberg false detection rate method was applied, with an alpha of 0.05.

6.3 Ethics

The study received a favourable opinion from the East of England (Cambridge Central Research) Ethics Committee (reference: 13/EE/0104). Approval was also obtained from Cambridge University Hospitals NHS (CUH) Trust, Cambridgeshire and Peterborough NHS Trust, Norfolk and Suffolk NHS Foundation Trust and North Essex NHS Foundation Trust, Research and Development departments.

Potential participants identified, as described above, who showed a willingness to take part in the research were provided with information about the study in the form of a patient information sheet. Following a period of time to consider the information, a follow-up phone call was made to inquire as to their interest in participation and to ask for further information to ensure they were eligible to take part. An appointment was then made at the study premises or at their home to provide an opportunity to ask further questions and obtain formal written informed consent from the participant or, in cases where the participant did not have capacity, from an appropriate consultee in accordance with the Mental Capacity Act 2005 (England and Wales). Consent was for participation in the study and publication of findings. Appendix 4 contains an example consent form and patient information sheet for this study.

All data collected for the study was kept securely with imaging data stored on security protected computer systems, accessible only to authorised users with log-in identities and passwords, on University of Cambridge servers, within the Wolfson Brain Imaging Centre and University of Cambridge Medical School and CUH Department of Radiology servers. Administration and neuropsychology test data was stored in paper form under lock-and-key and in computer-readable form in encrypted volumes securely hosted on the University of Cambridge Medical School servers.

The study was also ARSAC (Administration of Radioactive Substances Advisory Committee) approved.

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Aspects of the methodology in this chapter have been published in the study protocol paper in BMJ Open in January 2017 (Bevan-Jones *et al.*, 2017) where I was co-lead author.

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Chapter Seven

Inflammation in dementia with Lewy bodies – Results

7.1 Demographics

There were no differences in age or gender between the dementia with Lewy bodies (DLB) group and the two control groups (both the smaller ^{11}C-PK11195 (PK11195) cohort and the expanded cytokine cohort (see Table 7.1)), though the DLB group had fewer years of formal education than the two control groups. As expected DLB participants had lower cognitive scores as measured by the Addenbrooke's cognitive exam-revised (ACE-R) and mini mental state examination (MMSE) and higher motor scores on the unified Parkinson's disease rating scale - section III (UPDRS) compared to the control participants.

Table 7.1 Participant Demographics.

	DLB (n=19)	Control Group – PET Imaging (n=16)	Control Group – Cytokines (n=26)	Group Difference (DLB v Control Group - PET)	Group Difference (DLB v Control Group – Cytokines)
Gender (males/females)	15/4	8/8	15/11	*P*=0.09	*P*=0.20
Age in years: mean (± SD)	73.0(± 6.1)	70.0 (± 6.5)	69.9(± 6.4)	t=1.4; *P*=0.17	t=1.6; *P*=0.11
Education in years: mean (± SD)	11.7(± 1.9)	14.1(± 3.0)	14.7(± 2.8)	t=-2.9; *P*=0.007	t=-4.1; *P*=<0.001
MMSE scores: mean (± SD)	21.9(± 4.5)	28.9(± 1.1)	29.1(± 0.9)	t=-6.7; *P*<0.001	t=-6.9; *P*<0.001
ACE-R scores: mean (± SD)	65.7(± 12.9)	92.5(± 5.6)	94.0(± 5.0)	t=-8.2; *P*<0.001	t=-9.1; *P*<0.001
UPDRS scores: mean (± SD)	32.5(± 20.6)	N/A	N/A		
Disease duration in years: mean (± SD)	4.2(± 2.7)	N/A	N/A		
^{11}C-PK11195 PET scan	19	16	16		
^{11}C-PiB PET scan	16	0	0		

The control groups for Position Emission Tomography (PET) and cytokines (the latter consisting of 10 additional participants) were not significantly different to the DLB group with respect to gender and age. SD = standard deviation. ACE-R = Addenbrooke's Cognitive Examination-Revised. MMSE = Mini-Mental State Examination. PiB = Pittsburgh B Compound.

7.2 Cytokine Results

A total of 41 cytokines were assayed. Nine were removed from the analysis as follows. In three assays (interleukin (IL)-34, IL-23, IL-1β) there were no cytokines detected in any sample. In three further assays (IL-17A Gen B assay, IL-21, IL-31), between 1 and 3 samples had detectable levels - all within DLB subjects. For a further three assays less than a third of subjects in both groups had detectable levels (IL-1α, IL-5 and IL-4). Additionally, in two subjects there was a greater than 20% variation when the assay was duplicated (one DLB IL-17A assay and one control IL-12p70 assay), hence the mean for the group was substituted. Where a cytokine assay result was below the detectable threshold, zero was substituted in as the result.

7.2.1 Repeated-Measures General Linear Model

The repeated measures general linear model found no main effect of group ($F(1,41) = 0.24$, $P=0.63$) however a significant group by cytokine interaction was found $F(12,500) = 1.92$, $P =0.029$ following Greenhouse-Geisser correction ($\varepsilon=0.39$), (as Mauchly's test of sphericity was significant ($P<0.001$) indicting that variances of the differences were not equal). Post-hoc ANCOVAs of each cytokine, with age and gender as co-variates, showed that macrophage inflammatory protein 3α (MIP-3α) (F (41,1) = 13.29, P =0.001), IL-17A (F (41,1) = 7.75, P =0.008), and IL-2 (F (41,1) = 4.23, P =0.046) were higher in DLB and that IL-8 (F (41,1) = 5.46, P =0.024) was lower (see Table 7.2 for remaining results).

Table 7.2 Cytokine Results

Cytokine (all pg/ml) except where stated	Control Participants		DLB Participants		Log10 (x+1) transform		
	Mean	Standard Error	Mean	Standard Error	F	*P* value	% difference in means
IL-12p70	0.228	0.229	6.06	25.7	0.732	0.397	2553%
IL-2	0.140	0.218	0.496	0.637	4.234	*0.046	254%
IL-22	0.907	0.805	1.91	3.62	0.867	0.357	111%
IL-17A	3.04	1.58	5.67	6.19	7.747	*0.008	87%
MIP-3a	3.09	2.59	5.74	3.27	13.298	**0.001	86%
YKL-40	43034	28357	64150	46616	1.821	0.185	49%
IP10	324	147	434	368	0.976	0.329	34%
IL-12	151	119	174	90.6	1.894	0.176	15%
TNFR1 (CD120a)	3186	949	3527	737	1.446	0.236	11%
TNF α	2.92	1.02	3.16	0.99	0.033	0.856	9%
IL-7	17.5	5.55	18.7	8.55	0.139	0.711	7%
MCSF1	358	319	380	181	0.720	0.401	6%
TNF-β	0.367	0.139	0.385	0.213	0.061	0.807	5%
IL-27	2590	1421	2615	934	0.042	0.839	1%
IL-6	0.975	0.704	0.974	0.531	0.163	0.689	0%
IL-16	211	64.0	209	79.3	0.874	0.355	-1%
IL-15	2.46	0.812	2.41	0.325	0.033	0.856	-2%
hsCRP mg/l	3.19	5.05	3.10	3.79	0.001	0.981	-3%
GM-CSF	0.588	0.371	0.567	0.246	0.010	0.920	-3%
MCP-1	282	100	268	81.3	0.004	0.951	-5%
Eotaxin	181	52.4	171	83.0	1.726	0.196	-6%
MIP1a	17.1	9.08	15.7	4.78	0.812	0.373	-8%
MIP1b	130	74.6	119	35.8	0.158	0.693	-8%
IL-10	0.463	1.10	0.420	0.281	0.550	0.463	-9%
IFN gamma	11.4	8.19	10.2	7.26	0.503	0.482	-10%
MCP-4	197	59.4	175	58.8	1.498	0.228	-11%
Eotaxin 3	21.0	7.51	18.6	5.90	2.255	0.141	-11%
IL-13	0.594	0.749	0.512	0.697	0.723	0.400	-14%
TARC	320	241	272	146	0.094	0.761	-15%
IL-8	11.9	7.75	8.83	2.44	5.455	*0.024	-26%
VEGF	178	115	116	56.3	1.455	0.235	-35%
MDC	1644	2290	1018	161	0.899	0.349	-38%

Results of post-hoc ANCOVAs with age and gender as covariates of no interest (*=significant at *P*<0.05, **=significant at *P*<0.005). Cytokines are ordered according to differences in means between groups, with those cytokines highest in the DLB participants at the top. Abbreviations: IL = interleukin, TNF = tumour necrosis factor, MIP = macrophage inflammatory protein, MCP = monocyte chemotactic protein, MCSF = macrophage colony-stimulating factor, hsCRP = high sensitivity c-reactive protein, GM-CSF = granulocyte-macrophage colony-stimulating factor, IP-10= interferon gamma-induced protein 10, IFN = interferon, TARC = thymus- and activation-regulated chemokine, TNFR = tumour necrosis factor receptor, MDC = macrophage derived chemokine, VEGF = vascular endothelial growth factor. Showing four cytokines were significantly different between the groups. The largest difference in means was observed in IL-12p70, but one result in the DLB group was a high outlier, meaning overall there was no significant difference.

7.2.2 Support Vector Machine Analysis

With the support vector machine (SVM) model, peak accuracy for the classification of subjects based on cytokines was recorded at 81% (sensitivity of 71% and specificity of 87% in classifying DLB subjects correctly). MIP-3α, IL-8, IL-2, IL-13, vascular endothelial growth factor, YKL-40 (Chitinase-3-like protein 1) and IL-16 made up this discriminatory panel of cytokines. Cytokines were adjusted for age and gender prior to analysis by SVM.

7.3 PET Imaging Results

7.3.1 Repeated-Measures General Linear Model: DLB v Controls

In the repeated measures general linear model between the control and DLB groups, there was no statistically significant main effect of group ($F(30,1) = 0.13$, $P = 0.91$) or any significant group by region of interest interaction ($F(8,238) = 1.48$, $P = 0.165$; (Greenhouse-Geisser correction ($\varepsilon = 0.20$) as Mauchly's test of sphericity was significant ($P<0.001$)).

In view of the priori hypothesis, based on previous literature in PD and AD, that there would be greater inflammation in early stage DLB, the DLB group was split according to their median ACE-R score, resulting in nine "mild" cases with an ACE-R of >65 (Mild

DLB group) and ten “moderate-severe” cases with an ACE-R of ≤65 (Moderate-Severe DLB group), reflecting levels of cognitive impairment at the time of their PK11195 scans.

All three groups (Mild and Moderate-Severe DLB, plus controls) were similar in gender and age (see Table 7.3). Education was also similar between the Mild and Moderate-Severe DLB groups, but both DLB groups’ years of education were lower than controls. As expected, MMSE and ACE-R scores were significantly different between each of the three groups. Disease duration and UPDRS scores were higher in the Moderate-Severe DLB group than the Mild DLB group, but this was not statistically significant.

Table 7.3 DLB Subgroup Demographics.

	Mild DLB (n=9)	Moderate-Severe DLB (n=10)	Control Group – PET Imaging (n=16)	Group Difference (Mild DLB v Control - PET)	Group Difference (Moderate-Severe DLB v Control – PET)	Group Difference (Mild DLB v Moderate-Severe DLB)
Gender (males/females)	6/3	9/1	8/8	*P*=0.68	*P*=0.09	*P*=0.30
Age in years: mean (± SD)	74.7(± 5.2)	71.5(± 6.7)	70.0 (± 6.5)	t=1.87; *P*=0.07	t=0.55; *P*=0.59	t=1.17; *P*=0.26
Education in years: mean (± SD)	11.8(± 1.9)	11.7(± 2.1)	14.1(± 3.0)	t=-2.2; *P*=0.04	t=-2.3; *P*=0.03	t=0.09; *P*=0.93
MMSE scores: mean (± SD)	25.9(± 2.7)	18.3(± 1.8)	28.9(± 1.1)	t=-4.1; *P*<0.001	t=17.2; *P*<0.001	t=7.4; *P*<0.001
ACE-R scores: mean (± SD)	77.4(± 6.2)	55.2(± 6.2)	92.5(± 5.6)	t=-6.2; *P*<0.001	t=15.9; *P*<0.001	t=7.8; *P*<0.001
UPDRS scores: mean (± SD)	28.2(±14.4)	36.4(±25.1)	N/A	N/A	N/A	t=-0.86; *P*=0.40
Disease duration in years: mean (± SD)	3.0(±0.7)	5.2(±3.4)	N/A	N/A	N/A	t=-1.9; *P*=0.07
^{11}C-PK11195 PET scan	9	10	16			
^{11}C-PiB PET scan	9	7	0			

The Mild and Moderate-Severe DLB groups were similar to the control group, as well as each other, with respect to gender and age. There were differences in education between the Mild and Moderate-Severe DLB groups and the controls but not each other. As expected, MMSE and ACE-R scores were significantly different between each of the three groups. Disease duration and UPDRS scores were however not significantly different between Mild and Moderate-Severe DLB

participants.

7.3.2 Repeated-Measures General Linear Model - DLB Subgroups v Controls

Repeated measures general linear model analysis of these three groups, with age, gender and education as covariates, showed a significant main effect of group ($F(2,29) = 5.88$, $P=0.007$), and a main effect of region ($F(8,231) = 2.1$, $P=0.04$), but no group by region interaction $F(16,231) = 1.35$, $P =0.171$ (Greenhouse-Geisser corrections ($\varepsilon=0.198$) to the degrees of freedom were required as sphericity was violated ($P<0.001$)). Pairwise comparisons showed the main group effect was due to a significant difference between the Mild and Moderate-Severe DLB groups ($P=0.006$).

Post-hoc ANCOVAs with the same co-variates found 18 out of 41 regions to be significantly different between all three groups: caudate nucleus, cuneus, putamen, fusiform gyrus, lateral occipital lobe, inferior frontal gyrus, superior frontal gyrus, middle and inferior temporal gyrus, central superior temporal gyrus, anterior superior temporal gyrus, posterior temporal lobe, lateral orbital gyrus, anterior orbital gyrus, posterior orbital gyrus, inferolateral parietal lobe, superior parietal gyrus, thalamus and midbrain. The caudate nucleus (F (29,2) = 12.702, P =0.0001) showed the highest level of significance (see Fig. 7.1).

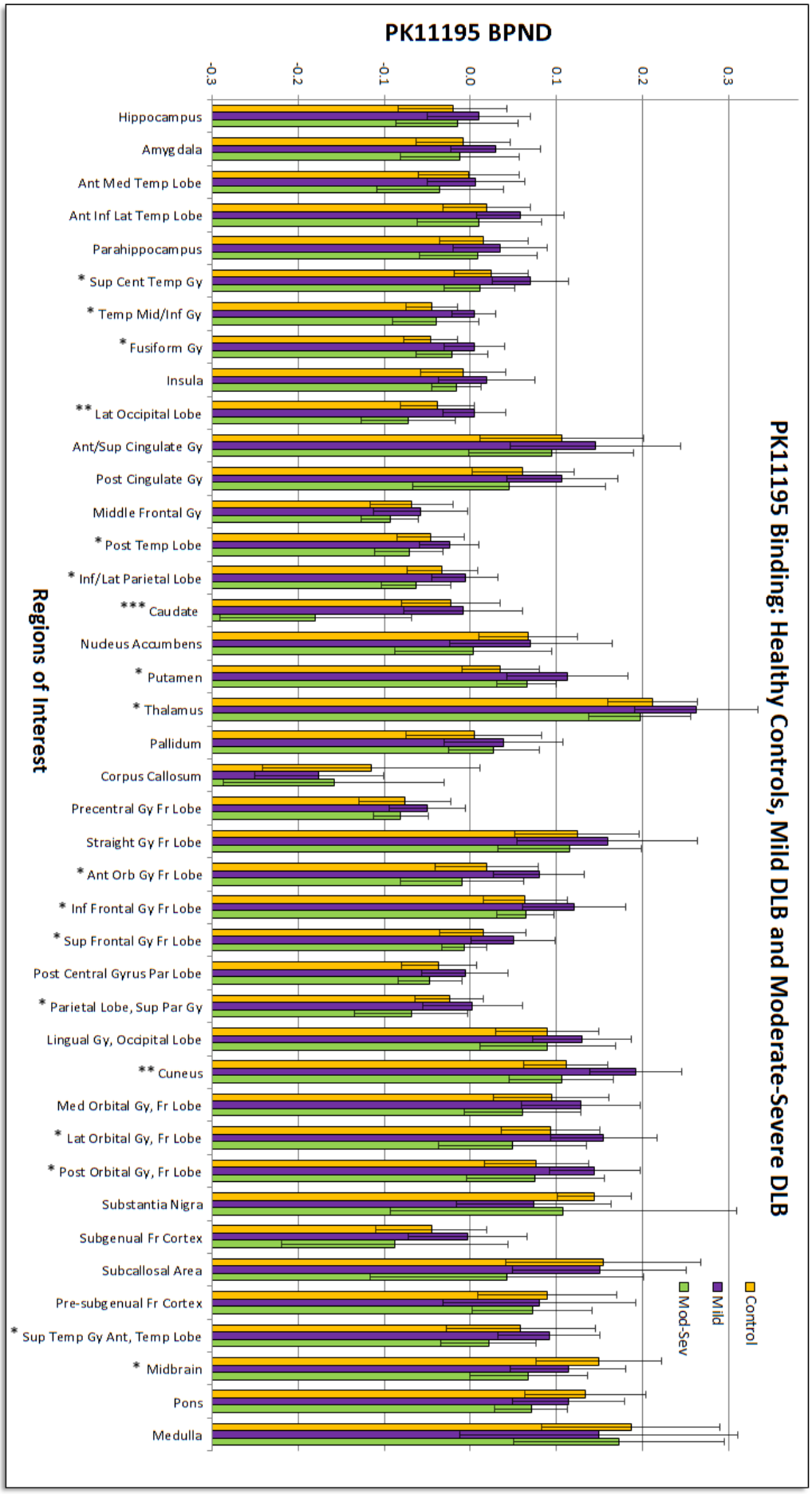
PK11195 BPND
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Hippocampus
Amygdala
Ant Med Temp Lobe
Ant Inf Lat Temp Lobe
Parahippocampus
* Sup Cent Temp Gy
* Temp Mid/Inf Gy
* Fusiform Gy
Insula
** Lat Occipital Lobe
Ant/Sup Cingulate Gy
Post Cingulate Gy
Middle Frontal Gy
* Post Temp Lobe
* Inf/Lat Parietal Lobe
*** Caudate
Nucleus Accumbens
* Putamen
* Thalamus
Pallidum
Corpus Callosum
Precentral Gy Fr Lobe
Straight Gy Fr Lobe
* Ant Orb Gy Fr Lobe
* Inf Frontal Gy Fr Lobe
* Sup Frontal Gy Fr Lobe
Post Central Gyrus Par Lobe
* Parietal Lobe, Sup Par Gy
Lingual Gy, Occipital Lobe
** Cuneus
Med Orbital Gy, Fr Lobe
* Lat Orbital Gy, Fr Lobe
* Post Orbital Gy, Fr Lobe
Substantia Nigra
Subgenual Fr Cortex
Subcallosal Area
Pre-subgenual Fr Cortex
* Sup Temp Gy Ant, Temp Lobe
* Midbrain
Pons
Medulla
Regions of Interest
PK11195 Binding: Healthy Controls, Mild DLB and Moderate-Severe DLB
Control
Mild
Mod-Sev

Figure 7.1 Group differences in PK11195 BP_{ND}. PK11195 BP_{ND} between controls, Mild and Moderate-Severe DLB - significant differences were found in 18 regions. ***=significant at *P*<0.001, **=significant at *P*<0.01 and *=significant at *P*<0.05. Error bars represent standard deviation. Abbreviations: med=medial, temp=temporal, ant=anterior, sup=superior, cent=central, inf=inferior, gy=gyrus, post=posterior, lat=lateral, par=parietal, orb=orbital, Mod-Sev=Moderate-Severe.

7.3.3 Comparison Between DLB Groups

Pairwise comparisons from the ANCOVAs found 14 individual regions had significant differences in binding between DLB groups, all with mean BP_{ND} higher in the Mild DLB group: superior and middle/inferior temporal gyri, anterior superior temporal gyrus, posterior temporal lobe, caudate, thalamus, anterior orbital gyrus, lateral orbital gyrus, inferior frontal gyrus, superior frontal gyrus, superior parietal gyrus, inferior lateral parietal lobe, cuneus, and lateral occipital lobe.

7.3.4 Comparison Between Control Group and Each DLB Group

In addition, comparing each DLB group with controls: 33 out of 41 regions showed higher binding in the Mild DLB group compared to controls (an exact sign test used to compare the differences found a significant increase in PK11195 BP_{ND} (non-displaceable binding potential) in the Mild group compared to controls, $P = 0.00004$), with five significantly higher (inferior and medial temporal gyrus, fusiform gyrus, putamen, inferior frontal gyrus and cuneus) in the ANCOVA. 33 out of 41 regions showed higher binding in the control group than the Moderate-Severe DLB Group (with an exact sign test finding a significant increase in the control group, $P=0.00004$), but only the caudate nucleus was significantly higher, when analysed as an individual region.

7.3.5 Support Vector Machine

Peak accuracy for the classification of DLB subjects from controls using the SVM model, based on PK11195 binding in regions of interest, was recorded at 75% (sensitivity of 68% and specificity of 84%) with the following five PK11195 regional BP_{ND} being the best for separating groups: caudate, putamen, midbrain, nucleus accumbens, and inferior frontal gyrus. For classifying Mild and Moderate-Severe DLB subjects, the peak accuracy was 83% (sensitivity 75% and specificity 89%), with the cuneus, lateral occipital lobe, caudate, superior frontal gyrus, anterior superior temporal gyrus and anterior orbital gyrus best for separating the subgroups. BP_{ND} values were adjusted for age, gender and education prior to analysis by SVM.

7.3.6 Amyloid status

Eleven of the sixteen “probable” DLB subjects who underwent PiB imaging, had standardized uptake value ratio (SUVR) >1.5, indicating positive amyloid status.

7.4 Correlation Analysis In DLB subjects

Pearson’s correlations were carried out between the clinical features (ACE-R score, UPDRS score and disease duration) and amyloid SUVR, together with log transformed cytokines and PK11195 BP_{ND} values in regions of interest. Cytokines significantly different in DLB as identified using the repeated measures general linear model were selected for correlation, in addition to the ten regions with the highest significant differences in the repeated measures general linear model between DLB groups.

ACE-R scores, were positively correlated with PK11195 BP_{ND} in four regions, with the caudate showing the strongest correlation (R=0.83, *P*=0.00008). Significant positive correlations were also found between ACE-R scores and binding in the cuneus (R=0.77, *P*=0.0005), superior frontal gyrus (R=0.69, *P*=0.003) and anterior orbital gyrus (R=0.67, *P*=0.004). Whilst correlations with ACE-R scores in the remaining six regions were non-significant, all showed positive correlations, ranging from R=0.25 to R=0.63 (see Fig. 7.2).

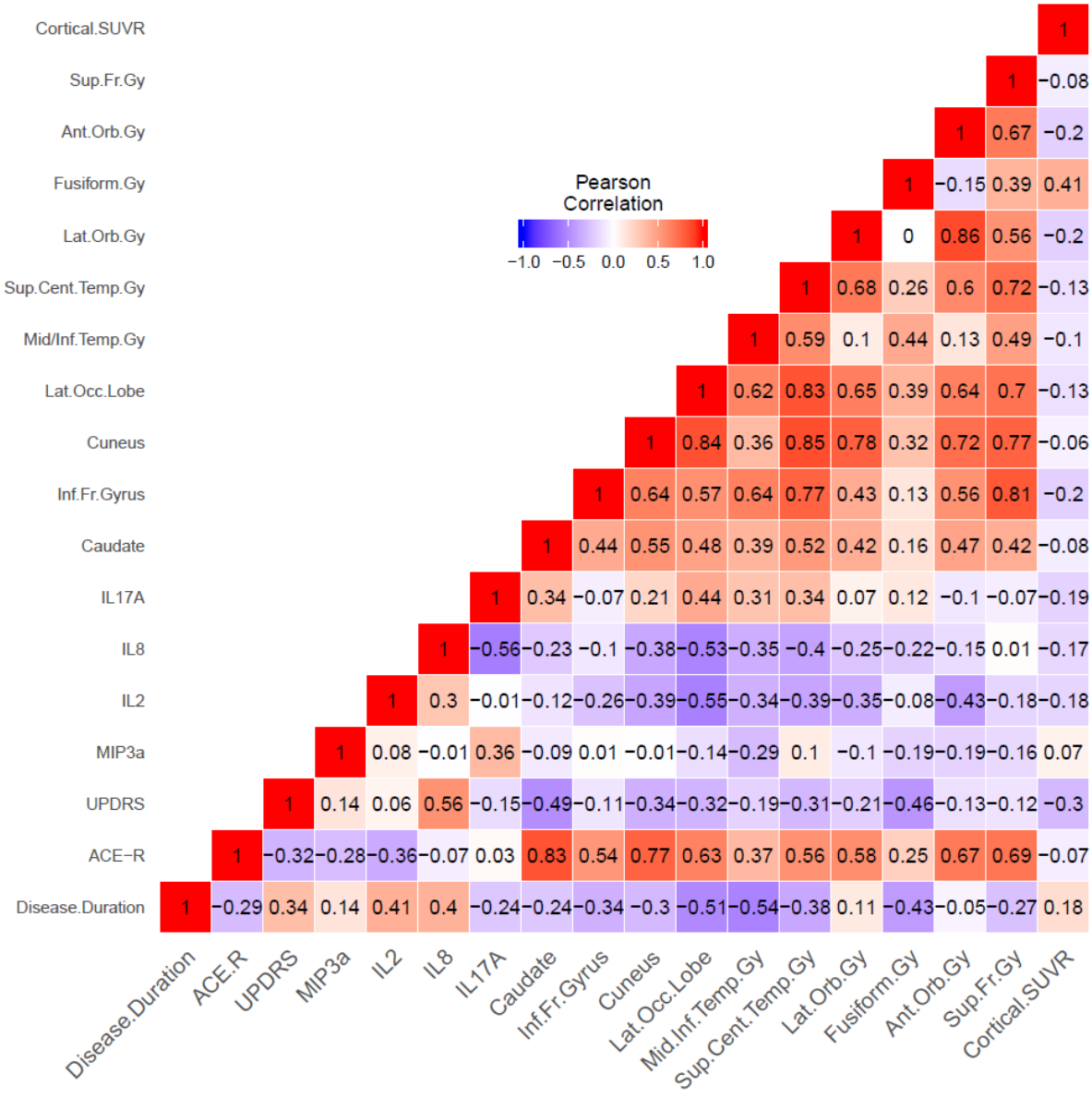

Figure 7.2 Clinical, Imaging and Cytokine Correlations. Pearson's partial correlations within the DLB group between clinical features, cytokines (log transformed) and PK11195 binding regions identified in the DLB group by the repeated measures general linear model. Age, gender and education were used as covariates. Abbreviations: temp=temporal, ant=anterior, sup=superior, cent=central, inf=inferior, gy=gyrus, lat=lateral, orb=orbital.

There were also negative correlations between inflammatory cytokines IL-2 and IL-8 and BP_{ND} in the lateral occipital lobe: R=-0.55, *P*=0.03 and R=-0.53, *P*=0.04, respectively and between the caudate BP_{ND} and UPDRS scores (R=-0.49, *P*=0.05), though these did not survive correction for multiple comparisons, see Fig. 7.3.

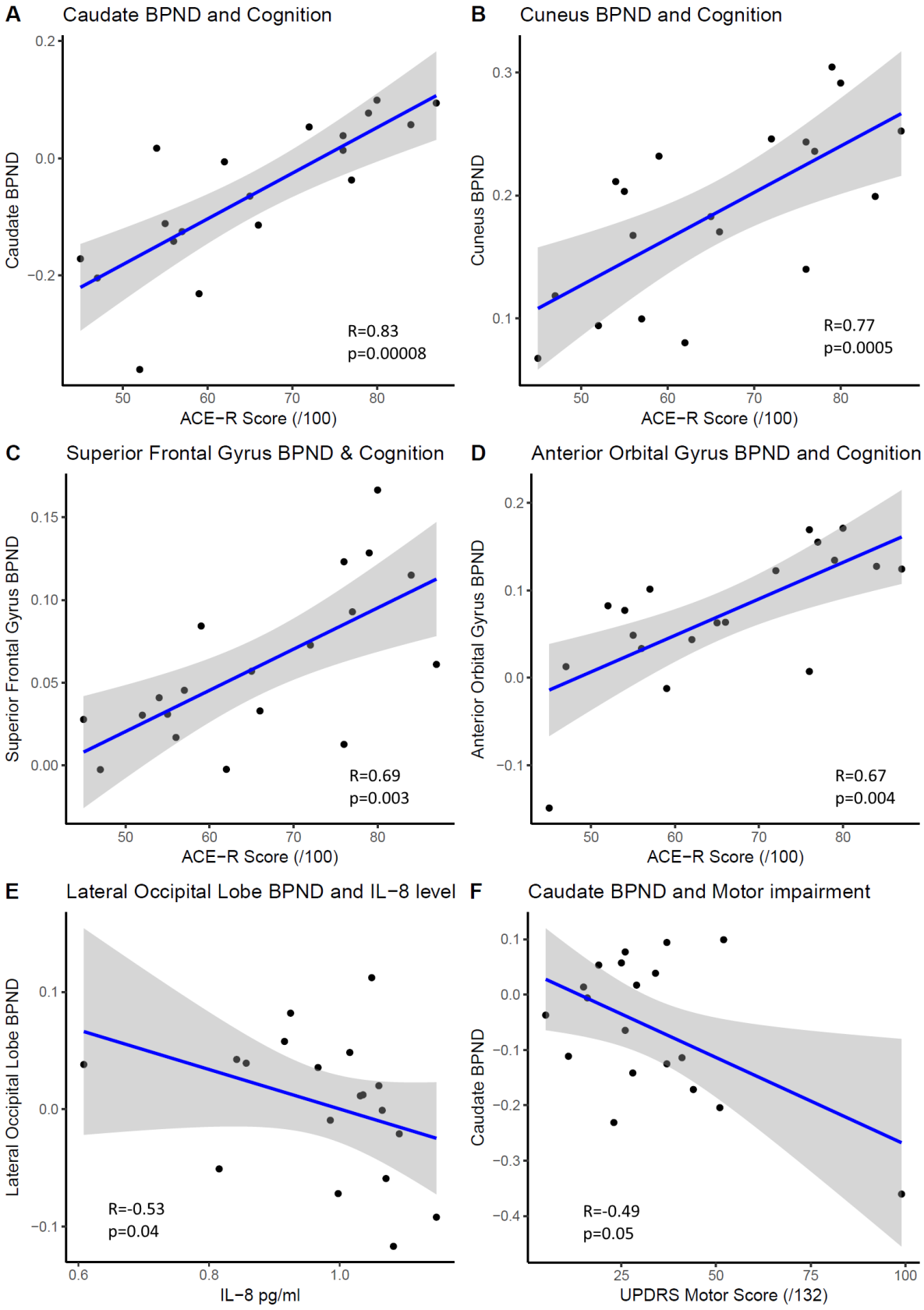

Figure 7.3 Associations between clinical and inflammatory markers. A-D show strong positive associations between cognition and regional PK11195

binding. E shows a negative association between central inflammation in the occipital cortex and peripheral inflammation in the form of IL-8 levels in the blood. F shows a negative association between the caudate and motor performance as measured by UPDRS. A-D, but not E and F, were statistically significant after correction for multiple comparisons.

Comparison of PK11195 binding in the caudate with disease duration and UPDRS scores, appeared to show that higher levels were associated with the Mild DLB group irrespective of disease duration and motor impairment (see Fig. 7.4). Further comparison of PK11195 binding in the caudate region and levels of MIP-3α, showed that low levels of caudate binding and high levels of MIP-3α appeared to be associated with severe DLB (see Fig. 7.5).

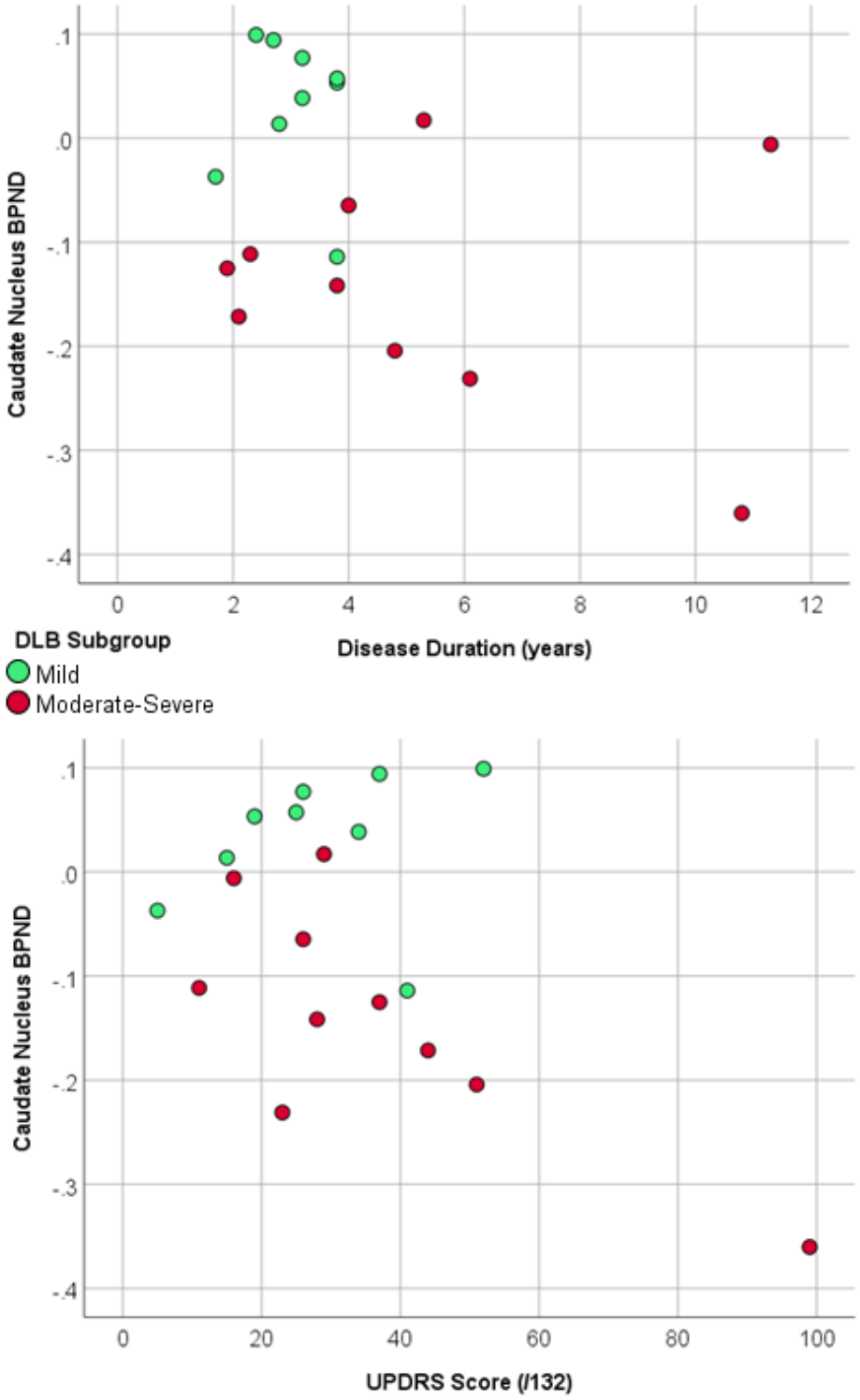

Figure 7.4 Clinical features and Caudate BP_{ND}. Comparison of caudate BP_{ND} with disease duration and UPDRS scores in the two DLB subgroups, showing that the association between microglial activation and the caudate was independent of disease duration and motor impairment.

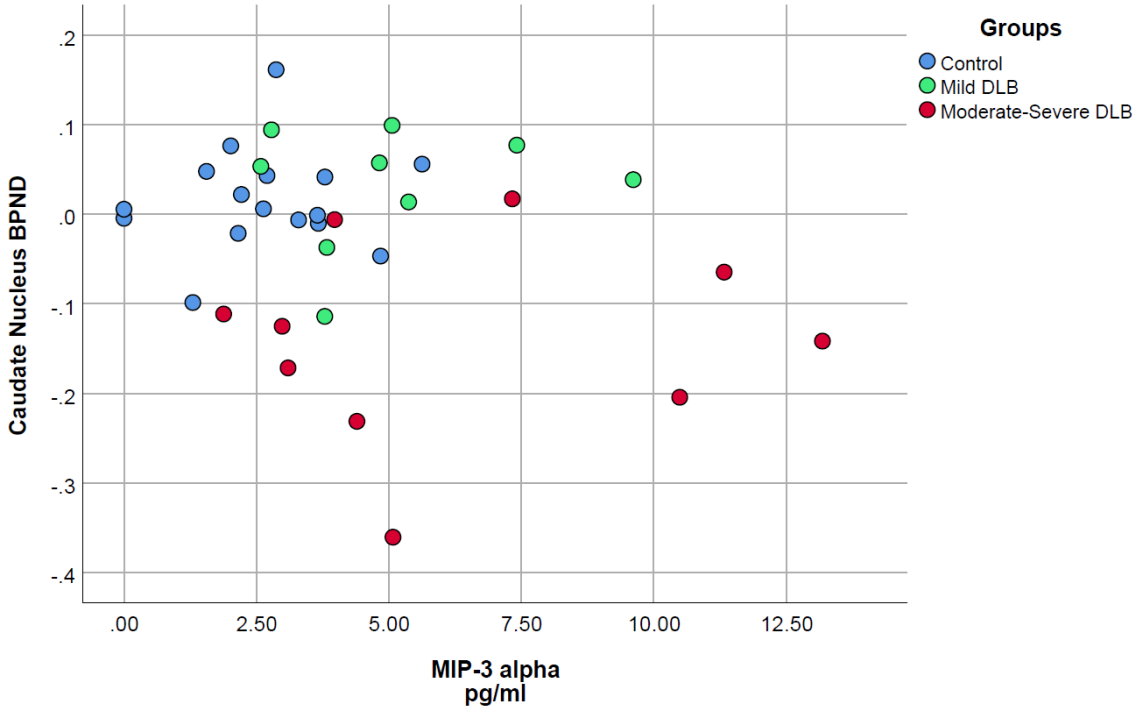

Figure 7.5 Caudate BP_{ND} and MIP-3α. Comparison of caudate BP_{ND} and MIP-3α levels in controls and the two DLB subgroups.

There was however no association between the level of PK11195 binding in the cuneus and the likelihood of the patient experiencing visual hallucinations (see Fig. 7.6). A comparison of binding potentials in the cuneus in DLB patients with and without visual hallucinations found no significant difference (t=-0.59, *P*=0.56)

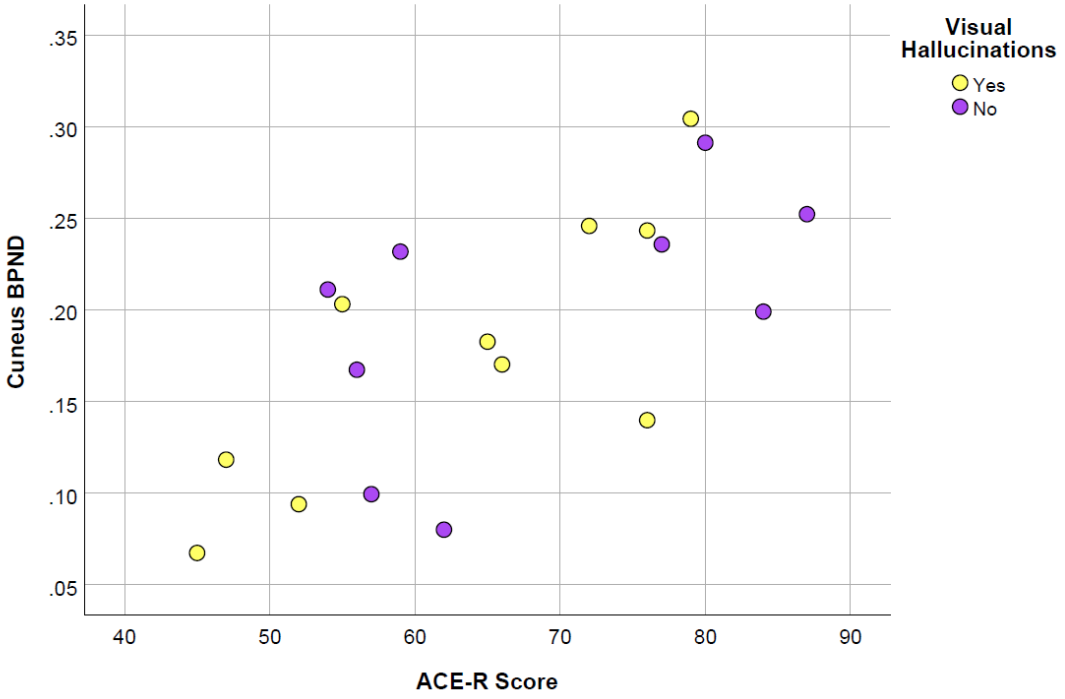

Figure 7.6 Cuneus BP_{ND}, ACE-R Scores and Visual Hallucinations. Despite the correlation between PK11195 binding in the cuneus and cognitive performance, there does not appear to be a link between level of binding and the likelihood of visual hallucinations being experienced by the patient.

Chapter Eight

Inflammation in Dementia with Lewy bodies – Discussion

8.1 Introduction

This study provides evidence of both central and peripheral inflammation in dementia with Lewy bodies and establishes a correlation between inflammation and clinical severity.

8.2 Evidence of Early Central Inflammation in DLB

^{11}C-PK11195 (PK11195) binding, which is a marker of microglial activation, was found to be significantly elevated in dementia with Lewy body (DLB) cases with mild disease compared to those who had moderate-severe disease. Healthy adults had lower levels of inflammation than mild DLB cases, but higher levels than those with moderate-severe DLB, indicating a non-linear spectrum of microglial elevation, starting early in the condition or before and subsiding in the late stages. The strong association found between cognitive scores and microglial activation in several brain regions (caudate, cuneus, anterior orbital gyrus and superior frontal gyrus), as well as a positive correlation in all the regions tested, is consistent with such a continuum.

Early microglial activation is consistent with results in prodromal and early Alzheimer's disease where positron emission tomography (PET) studies have previously shown microglial activation in mild-cognitive impairment, before the onset of dementia (Okello *et al.*, 2009) and early in dementia with a similar correlation with cognitive performance by means of mini mental state examination (MMSE) score (Hamelin *et al.*, 2016). Inflammation has also been seen early in Parkinson's disease in the brainstem, and cortical inflammation is seen before the onset of dementia (Ouchi *et al.*, 2005; Gerhard *et al.*, 2006; Edison *et al.*, 2013). In addition, inflammation has been reported even earlier, in subjects with no motor or cognitive impairment, only REM sleep behaviour disorder, a condition which is recognised as a prodromal stage of synucleinopathies, within the substantia nigra (Stokholm *et al.*, 2017), and occipital cortex (Stokholm *et al.*, 2018).

From this study, we cannot conclude at what point in the DLB process inflammation occurs, only that it is already present in mild disease. It remains possible subjects predisposed to develop DLB have lifelong increases in activated microglia, though from mouse models of a number of dementia subtypes, which suggest activation is a response to excess protein or protein accumulation (Schwab, Klegeris and McGeer, 2010), this seems unlikely. Early neuroinflammation that then plateaus has been reported in a

mouse model of Alzheimer's disease (López-Picón *et al.*, 2017), but to date a decline in central inflammation with disease progression has not been reported in human studies. Other studies in Alzheimer's disease have reported an early and late peak (Fan *et al.*, 2017) (though these peaks were not demonstrated in the same subjects) or an increase in inflammation with disease progression (Fan, Okello, *et al.*, 2015)(Hamelin *et al.*, 2018) but in only eight or six (respectively) demented Alzheimer's subjects who individually had heterogeneous (both rises and falls in inflammation) on follow-up scanning. In addition, in Parkinson's disease an inverse relationship between microglial activation and MMSE scores has also been found (Fan, Aman, *et al.*, 2015), suggesting caution is indicated in the interpretation of this study's result. This study provides cross-sectional data only and longitudinal studies are required to study the role of central inflammation as impairment progresses.

The strength of the association between cognition and inflammation was highest in the caudate, which was also identified by the support vector machine (SVM) as a key classifier in determining differences between the control and DLB groups. The caudate is a core anatomical area involved in the pathology of DLB and caudate dysfunction could be caused by defects in the nigrostriatal pathway (Minoshima *et al.*, 2002) and/or their targets, the medium spiny neurons (Zaja-Milatovic *et al.*, 2006), both of which show selective degeneration in DLB, compared to Alzheimer's disease. Early inflammation of the caudate, which declines with cognitive impairment, implicates a key component of the basal ganglia in the cognitive impairment seen in DLB. Indeed, hypometabolism in the caudate of DLB patients has previously been detected in early disease (Huang *et al.*, 2015). In addition, in a comparison with Parkinson's disease patients, DLB patients are reported to have a more severe reduction in dopamine uptake within the caudate (Walker *et al.*, 2004; Gomperts *et al.*, 2016). The caudate has extensive cortical (as well as nigrostriatal) inputs, and is increasingly recognised for its role in higher cognition, particularly executive function and goal-directed action (Grahn, Parkinson and Owen, 2008; Haber, 2016).

A strong correlation between cognitive scores and PK11195 binding in the occipital lobe, within the cuneus, was also found, but no link between the level of binding and visual hallucinations being experienced by the patient. Occipital lobe pathology has been frequently reported in DLB, with both Fluorodeoxyglucose (FDG) PET and perfusion Single Photon Emission Tomography (SPECT) scans showing reduced metabolism (Minoshima *et al.*, 2001; Kantarci *et al.*, 2012) and perfusion (Yeo *et al.*, 2013) respectively, distinguishing DLB cases from those with Alzheimer's disease.

Visuospatial dysfunction is also a specific indicator of DLB pathology (Yoshizawa, Vonsattel and Honig, 2013). Our results suggest inflammation maybe linked to the underlying pathology of these cognitive impairments.

Furthermore, higher levels of microglial activation in the caudate appeared to be associated with milder cognitive impairment independently of disease duration or level of motor impairment, which may indicate a stronger link between caudate dysfunction and cognitive performance in DLB and a protective effect of microglial activation, at least initially. A longitudinal study in brain trauma patients found evidence of a protective role for microglia clinically, with the drug minocycline reducing microglial activation over 12 weeks, with an associated increase in neurodegeneration (Scott *et al.*, 2018). However, early microglial activation that is then primed by systemic inflammatory factors towards chronic and deleterious inflammation has also been suggested as a potential mechanism in neurodegeneration (Perry and Holmes, 2014).

8.3 Evidence of Peripheral Inflammation in DLB in Comparison to Controls

As well as central inflammation, we report increased peripheral cytokines in DLB subjects as a whole compared to controls. A significant cytokine by group interaction was found, suggesting individual cytokines had a different effect depending on the group that they were in. DLB participants showed higher levels of macrophage inflammatory protein – 3α (MIP-3α), interleukin (IL)-17A, IL-2 and lower levels of IL-8 in the serum compared to their healthy counterparts. MIP-3α, IL-2 and IL-8 were also identified by SVM as classifiers in separating the DLB group from controls. SVM was able to differentiate the groups with an accuracy of 81%, suggesting cytokine profiles between controls and DLB patients were indeed different.

MIP-3α, also known as CCL20, and IL-17A share a close relationship, with MIP-3α regulating helper T cells that produce IL-17a. IL-17a is strongly implicated in the pathogenesis of a number of autoimmune disorders. In rheumatoid arthritis in particular, IL-17a appears to promote a chronic pro-inflammatory state leading to bone and cartilage destruction (Schutyser, Struyf and Van Damme, 2003; Onishi and Gaffen, 2010; Lee and Körner, 2014) and levels have been found to fall following treatment of rheumatoid arthritis with monoclonal antibodies such as Infliximab (Kawashiri *et al.*, 2009). Whether these two cytokines play a destructive inflammatory role in DLB requires further investigation.

IL-2 has a number of anti-inflammatory and pro-inflammatory roles within the immune system, but predominantly is a marker of T-cell activation (Boyman and Sprent, 2012), again suggesting a role for T-cells in DLB pathology. IL-8 is a mediator of inflammation through recruitment and degranulation of neutrophils, and can also promote phagocytosis in neutrophils (Waugh and Wilson, 2008).

Only one prior study has investigated peripheral cytokine levels in DLB (King *et al.*, 2017). No differences were found compared to healthy controls, however MIP-3α, IL-17a and a large number of cytokines that were included in this current study were not investigated. IL-2 was not found to be raised in the DLB group but was raised in the prodromal DLB group and no differences in IL-8 results were found in either cohort. In Alzheimer's disease, a systematic review of peripheral inflammatory markers showed IL-2 but not IL-8 was consistently raised (Lai *et al.*, 2017). MIP-3α and IL-17a were not mentioned in that review. Mouse models of Alzheimer's disease however suggest T-helper cell infiltration into the brain parenchyma is combined with elevated IL-17 levels in the serum, cerebrospinal fluid and hippocampus in association with amyloid pathology (Zhang *et al.*, 2013). T-cells have also been implicated in the pathology of Alzheimer's, through the IL-17 pathway (Sommer, Winner and Prots, 2017).

8.4 Correlations between Central and Peripheral Inflammation

Combined low levels of PK11195 binding in the caudate and higher levels of MIP-3α were associated with moderate-severe DLB, and hint at a link between falling central inflammation and rising peripheral inflammation, involving the adaptive immune system. In addition, a negative, though non-significant, association was found between IL-2 and IL-8 levels and PK11195 binding in the occipital lobe.

Rising systemic inflammation could be associated with a fall in central inflammation. It is possible that microglia switch from being protective initially to destructive as the disease progresses - leading to the initiation of a peripheral response - before subsiding as neuronal death follows. There are suggestions that MIP-3α is released when microglia are in their pro-inflammatory (rather than anti-inflammatory state) in association with the release of free radicals (Orihuela, McPherson and Harry, 2016), but the evidence is limited. Further studies looking at this potential interaction are required.

8.5 No Correlations with Amyloid

We did not find any correlation between amyloid load and clinical features, binding in the regions of interest or peripheral cytokine levels, suggesting either that amyloid is not a driver of inflammation or disease in DLB, or that any such association is weak. This is in contrast to other studies which have shown a local or regional correlation between amyloid and inflammation in Alzheimer's disease (Fan, Okello, *et al.*, 2015; Hamelin *et al.*, 2016; Parbo *et al.*, 2017) and to a lesser extent in Parkinson's disease dementia (Fan, Aman, *et al.*, 2015).

8.6 Strengths and Limitations of this Study

The strengths of this study include the novel use of the combined methodologies of PET imaging, serum inflammatory profiles and clinical assessments to assess the extent and nature of inflammation in DLB subjects. There are no studies in the literature which have correlated peripheral and central inflammation in the same dementia subjects.

Another strength is the use of a machine learning technique to add to the standard parametric analysis used to compare groups. The support vector machine results were consistent with the parametric test results with respect to showing differences between groups and identifying key classifiers, adding further validity to the results.

Potential limitations include the use of the PK11195 ligand as a measure of microglial activation, via binding to the peripheral benzodiazepine receptor. PK11195 is accepted to have a high signal to noise ratio and also displays non-specific binding, particularly to astrocytes (Vivash and OBrien, 2016). It nevertheless provides the best means currently available for assessing *in vivo* central inflammation as other ligands for the peripheral benzodiazepine receptor are affected by polymorphisms in the gene for the receptor (Owen *et al.*, 2012), which would mean that subject recruitment would be limited to those with moderate to high affinity binding, diminishing the number of potential DLB participants that could be recruited. This is relevant for DLB studies where patient recruitment is already made difficult by the low clinical prevalence rate, but may be less relevant for studies in Alzheimer's disease subjects.

Another potential limitation is the inference of evolutionary changes in a cross-sectional study such as this. Whilst the correlations with cognitive performance were strong and provide some evidence of a correlation with disease severity, they are not substitutes for

a longitudinal study of PK11195 binding in the same subjects assessing inflammatory changes over time.

The serum biomarker study tested a large number of inflammatory markers but was limited by the small sample size. However as this study was primarily focussed on the correlation between central and peripheral inflammation, the number of patients in the serum study was limited by the number who could carry out PET imaging.

8.7 Conclusion

Overall our results suggest that early DLB is associated with microglial activation in key areas affected by DLB pathology, which appears to decline as cognitive impairment progresses. Peripherally, cytokines associated with T-cell activation appear to be higher in DLB. The next step is for a longitudinal study of central and peripheral inflammation starting in early DLB, to understand if progressive disease is linked to both pathways, and hence if selectively targeting either could halt disease progression.

8.8 References

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Chapter 9

Conclusion and Future Work

9.1 Objectives and Summary of Results

This study aimed firstly to investigate the current clinical practice in relation to the recognition and diagnosis of Lewy body dementia, and the difficulties associated with both and then aimed secondly to investigate if inflammatory changes were present in Lewy body dementia, and whether these could thus assist in improving recognition and diagnosis of the condition. If present, inflammatory changes could potentially provide a biomarker to aid diagnosis or could potentially lead to a disease modifying therapy. Any novel treatment such as the latter could raise motivation to diagnose LBD and hence increase vigilance of these conditions clinically.

A background literature review of studies assessing prevalence of both dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD) found DLB forms 5% and PDD 3.6% of all clinically diagnosed dementia cases, meaning LBD accounts for 8.6% of all dementia cases. At autopsy however, this is a much higher 15-20%, suggesting a mismatch between clinical and pathological rates of LBD, where a half or more of LBD cases are not being diagnosed clinically. The rate of dementia in PD was found consistently to be 20-30% of PD cases.

Rates of diagnosis of both DLB and PDD in routine practice within NHS trusts in East Anglia were found to be even lower than previously reported clinical prevalence rates. Only 3.3% of dementia cases were diagnosed with DLB, lower than that expected from the literature review and only 8.3% of patients with PD were diagnosed with dementia, less than half of the expected rate from the review. This study intentionally looked at routine practice to identify the actual rates of these conditions diagnosed clinically reflecting patient experience, whereas the vast majority of studies are based on a diagnosis made by the study team, actively seeking to identify dementia subtypes. Hence this study reflects actual practice in the NHS within the UK and found that far fewer cases are being detected clinically than are reportedly found to be LBD at autopsy, suggesting that a large number of patients with LBD are misdiagnosed as another dementia subtype or alternatively are not specifically diagnosed with any dementia subtype.

Compared to patients with other dementia subtypes, DLB patients had more clinic appointments with more alternate diagnoses, prior to reaching a final diagnosis. In addition, the majority of patients who were diagnosed with DLB, exceeded the threshold for "probable" DLB, in terms of the core and suggestive features they experienced, as set out in the 2005 consensus criteria. In addition, only one DLB patient out of 23 had a dopamine transporter scan, suggesting this was a much under used biomarker.

In PD patients, delays were found between the onset of symptoms suggestive of dementia and an actual diagnosis and more than a quarter were started on treatment for dementia, in the form of rivastigmine, before being diagnosed. Carer stress was also higher in PD patients with dementia compared to those without, as were visual hallucinations and cognitive fluctuations.

Cognitive impairment was frequently found in the control PD subjects, who were not formerly diagnosed with dementia and some had even had treatment for dementia. This suggests once more that there are delays in the diagnosis of dementia in PD.

A literature review of studies examining inflammation in Lewy body dementia found imaging evidence of microglial activation early in disease in PD subjects and PDD subjects. However, pathological evidence in the form of autopsy studies in DLB found increased MHC class II expression, with some evidence of phagocytosis and dystrophic changes in microglia, but not hypertrophic change, at the latter stages of the disease. Over-expression of α-synuclein in mouse models, leads to microglial activation and neuronal loss, providing a substrate for a toxic inflammatory reaction, MHC class II molecules and toll like receptor (TLR) 2 appear to be key to this process. However a number of other inflammatory components are found to be neuroprotective in α-synuclein mouse models, including TLR 4, NF-E2-related factor 2 and fractalkine, hence the role of microglia in LBD and other synucleinopathies remains unclear. Cerebrospinal fluid and blood biomarker studies in DLB and PDD provide limited evidence of inflammation in these disorders.

In a study of ^{11}C-PK11195 (PK11195) PET imaging in DLB compared to controls, this study found increased PK11195 uptake in mild but not in moderate-severe cases of DLB, consistent with microglial activation *in vivo* occurring early in the disease process. The level of microglia activation in the caudate and cuneus correlated strongly (with an R of more than 0.7) with revised Addenbrooke's cognitive exam (ACE-r) scores in DLB subjects, again suggesting central inflammation occurs early in disease. Assessment of a panel of serum inflammatory biomarkers revealed several inflammatory changes (macrophage inflammatory protein-3, interleukin-17A and interleukin-2 were elevated, and interleukin-8 reduced), in the serum of DLB patients compared to controls. There was no correlation between cortical amyloid load as measured by ^{11}C Pittsburgh Compound B PET imaging in DLB and PK11195 uptake suggesting an association between central inflammation and cortical amyloid load. Nor was there a correlation between cortical amyloid load and clinical features or peripheral inflammatory markers.

9.2 Conclusions

9.2.1 Diagnosing DLB

In this study, the proportion of dementia cases given a diagnosis of DLB in clinical practice was much lower than studies suggest is the true prevalence of the disorder pathologically and also lower than previously reported clinical rates. Many dementia cases in routine clinical services were given a non-specific dementia diagnosis of either unspecified or mixed with no mention of possible underlying pathology. It is possible, and indeed likely, that some of these cases were DLB. Clinicians appear to find it difficult to diagnose DLB and have high thresholds for diagnosing the condition in practice, as evidenced by the fact that more clinic appointments and changes in diagnosis were made in subjects diagnosed with DLB, compared to non-DLB controls, prior to their final diagnosis and the majority of subjects who did receive the diagnosis exceeded the thresholds for "probable" DLB (McKeith *et al.*, 2005). It is recognised that the 2005 DLB criteria are highly specific but not very sensitive (Nelson *et al.*, 2010) (Huang and Halliday, 2013), hence opting to set a higher threshold than the DLB criteria is unlikely to improve the accuracy of the diagnosis. Whilst a sensitive and specific biomarker in the form of a dopamine uptake scan (Walker and Walker, 2009; Thomas *et al.*, 2017) does exist, it was rarely used. The reasons for this could include the high cost and poor availability. The criteria have been updated since this study was undertaken (McKeith *et al.*, 2017) and this may improve diagnosis rates. However the two new biomarkers of ^{123}Iodine- metaiodo-benzylguanidine (MIBG) myocardial scintigraphy and polysomnography which have been proposed may also be difficult to access and expensive, and hence may not provide what is needed: a biomarker that is easily available and specific yet also sensitive.

9.2.2 Diagnosing PDD

Compared to previous studies on the prevalence of dementia in PD, which consistently report between 20-30%, this study found a much lower rate in actual clinical practice. A possible reason for this difference could be a delay between the recognition of dementia symptoms and a formal diagnosis. This study also found that symptoms of dementia were noted in clinic, but the diagnosis was not made until later and treatment often started before a diagnosis, perhaps indicating an inability or reluctance to diagnose

dementia despite clinicians acknowledging that it was nevertheless present. Often patients were referred by neurologists and geriatricians to old age psychiatrists for diagnosis and the majority of dementia diagnoses were indeed made by old age psychiatrists, despite neurologists and geriatricians providing the majority of care through follow-ups. The importance of making the diagnosis however was highlighted by the increased presence of additional symptoms such as excessive daytime sleepiness, swallowing difficulties, repeated falls, anxiety, orthostatic hypotension and changes in personality in those with PDD together with increased levels of carer stress. In addition, a dementia diagnosis can open further avenues of support for patients and their carer-givers but perhaps more importantly may mean increased recognition that the patient's dementia has significant implications including loss of insight, poor judgement and poor financial decision making (Aarsland et al., 2001). Potential avenues to consider in order to increase the rates of formal dementia diagnosis include improved education of neurologists and geriatricians regarding dementia in PD and providing a biomarker of dementia.

9.2.3 Central Inflammation in DLB

DLB subjects also show evidence of increased PK11195 uptake, consistent with increased microglial activation in mild disease, and reduced levels in moderate to severe disease, suggesting that inflammation occurs early in the condition. A decline in cognitive performance was strongly associated with a fall in microglial activation in the caudate, a region which is recognised as important in the DLB pathology. Similarly, the cuneus in the occipital lobe, also showed a positive correlation as did many other regions. Early central inflammation is consistent with previous studies in PD and PDD plus in a small case series in DLB (Ouchi *et al.*, 2005; Gerhard *et al.*, 2006; Iannaccone *et al.*, 2013; Fan *et al.*, 2015). High levels of microglial activation in the caudate were associated with higher levels of cognitive performance irrespective of how long subjects had the disease and how severe their motor impairment was, suggesting that microglia may be playing a key role in either maintaining cognition through neuroprotection or being the instigators of cognitive decline where high levels of activity are seen before the onset of dysfunction. The case for both scenarios has been made previously (Perry and Holmes, 2014; Scott *et al.*, 2018), and it is not possible to make this distinction in a cross-sectional study such as this.

Comparison with autopsy studies is also difficult as most patients reaching autopsy are in the mid to late stages of disease, yet in small numbers of DLB subjects, previous pathological studies report a lack of hypertrophic change indicative of the classically defined activated pro-inflammatory microglia (Walker and Lue, 2015) within the temporal pole and superior frontal gyrus (Streit and Xue, 2016). Increased MHC class II expression, another marker of activation, have however been found, in the entorhinal cortex (Mackenzie, 2000) and amygdala (Togo *et al.*, 2001), together with evidence of dystrophic change in the hippocampus (Bachstetter *et al.*, 2015). The results of this study do not conflict with these pathological findings, as microglial activation was higher only in early disease. In addition PK11195 binding is a marker of activation and is not increased by further transformation of microglia into a hypertrophic or amoeboid shape (Banati, 2002). The results of this study also suggest that any future trials of anti-inflammatory therapy should only include mild cases, and that potential patients should have an ACE-R score of more than 65. In addition, inflammation in the milder stages of disease could mean targeted therapies are able to modify disease progression early and prevent further deterioration, highlighting the importance of unravelling the underlying mechanisms and their impact on disease. For this purpose, there is also a need to study inflammation even earlier, in the prodromal or pre-dementia stage of DLB.

9.2.4 Peripheral Inflammation in DLB

Inflammatory cytokines in the blood are also altered in DLB subjects as a whole compared to controls, with macrophage inflammatory protein-3, interleukin (IL) 17A and IL-2 elevated. All three are associated with T cell activation and hence recruitment of the adaptive immune system. The only prior study to have investigated serum inflammatory markers in DLB by King *et al*. found raised IL-2 in prodromal DLB, but not in established disease, macrophage inflammatory protein-3 and IL-17A were not tested (King *et al.*, 2017). There was no significant correlation between disease severity and IL-2 levels in the current study, hence this result needs further confirmation in a larger group of DLB patients. Acquired immune system involvement in the disease is consistent with increased MHC class II expression in microglia at autopsy as described earlier in DLB. If the adaptive immune system in the form of T cells are the final path in cell degeneration, they could represent a rich target for therapy. Drugs that attenuate this aspect of the immune system are already widely used clinically - in the neuroinflammatory disorder multiple sclerosis and include alemtuzumab, natalizumab, and glatiramer acetate (Martin

et al., 2016). T cell involvement in Lewy body disorders is supported by studies showing T cell infiltration in the substantia nigra of PD patients at post-mortem (Brochard *et al.*, 2009) and polymorphisms in HLA regions coding segments of the MHC class II molecule representing increased risk in PD (Hamza *et al.*, 2010; Wissemann *et al.*, 2013). Knocking out MHC Class II protein also prevents dopaminergic cell loss in mouse models of α-synuclein overexpression (Harms *et al.*, 2013).

IL-8 levels were lower in DLB subjects. IL-8 is a pro-inflammatory chemokine involved mainly in neutrophil recruitment and angiogenesis (Waugh and Wilson, 2008). It has also been reported to be secreted by microglia in response to pro-inflammatory stimuli (Ehrlich *et al.*, 1998). The reduction seen in this study is not consistent with results of the study by King and colleagues (King *et al.*, 2017) however, which found no differences in IL-8 between DLB subjects and controls. Hence this result also needs verification in larger studies.

9.2.5 Could Inflammation Provide a Biomarker for the Diagnosis of DLB?

This study supports the concept of identifying an accurate biomarker for DLB through inflammation. Both central and peripheral inflammation are detected in DLB, with central inflammation closely related to disease severity. Whilst this study provides evidence for the distinction of DLB from non-dementia subjects through inflammation, the next step is to identify variations between inflammatory pathways between DLB and other dementia subtypes and initially this must be focussed on Alzheimer's dementia (AD), the most common dementia and one which is often hard to differentiate from DLB. However differentiation from other parkinsonian disorders must also be considered in the search for specific biomarkers.

There is evidence to suggest that inflammation in AD and DLB have both common and divergent patterns, however the evidence for inflammation in AD is much better established than DLB, due to the much larger number of studies in AD, hence making comparisons is currently difficult. Early central inflammation as detected using ^{11}C-PK11195 PET imaging also occurs in AD and mild cognitive impairment (Okello *et al.*, 2009; Hamelin *et al.*, 2016), however in AD there is also a reported late peak (Fan *et al.*, 2017), albeit this latter study reported early and late increases in different AD subjects. At post-mortem, increased HLA- DR positive hypertrophic microglia are found in the hippocampus (McGeer *et al.*, 1988) and increased microglial density and fewer

dystrophic microglia compared to DLB has also been reported in the hippocampus (Bachstetter *et al.*, 2015), suggesting different trajectories of microglial activation once established. In AD, a negative correlation between PK11195 whole cortical gray matter binding and MMSE scores (Edison *et al.*, 2008) and binding in the precuneus and performance in delayed recall at 30 minutes (as measured by the Rey Auditory Verbal Learning Test) (Passamonti *et al.*, 2018) have also been reported. This is the opposite of that reported in DLB in the current study. Hence microglial activation could have different roles in these two conditions. It is possible to speculate that extracellular amyloid and intraneuronal tau could lead to a different inflammatory pathway compared to oligomeric alpha-synuclein and intraneuronal Lewy bodies.

A meta-analysis of peripheral inflammatory markers in AD, found elevations in several compared to controls, including IL-2, IL-6, high sensitivity CRP, soluble tumour necrosis factor receptor 1 and interferon-γ (Lai *et al.*, 2017). In contrast, only IL-2 was raised in the current study of DLB, not the others listed. Hence a larger study of inflammatory biomarkers in DLB and AD may reveal sufficient differences to allow differentiation of these conditions by peripheral inflammatory profile. The current study was able to differentiate DLB subjects from controls using a panel of seven inflammatory markers and application of a support vector machine, with a sensitivity of 71% and specificity of 87%, with just 19 DLB participants and 26 controls. Blood tests are less invasive and expensive than imaging and CSF analysis and would be a simple and accessible means of diagnosis. Attempts to create such a test using metabolites and pathogenic proteins in the blood in AD are already underway (Fiandaca *et al.*, 2015; Varma *et al.*, 2018). Nevertheless any such test would require careful validation and any potential confounders, such as lifestyle differences, concurrent medications and co-existing conditions, would need to be considered.

9.2.6 Potential Application to PDD

This study did not assess inflammation in PDD, because it has been previously studied with several reports of early microglial activation (Ouchi *et al.*, 2005; Gerhard *et al.*, 2006; Fan *et al.*, 2015). Conversely, only one study has investigated peripheral markers and found high sensitivity CRP was elevated in the serum of PDD patients compared to controls though tested only one other biomarker – fibrinogen (Song *et al.*, 2013). Hence further studies are required to investigate peripheral inflammatory markers. Nevertheless, the results of the current study are likely to be relevant to PDD. DLB and

PDD are indistinguishable pathologically, hence the inflammatory profile is unlikely to be vastly different. Comparison may however reveal the mechanisms behind the differing clinical presentations – potentially unravelling why dementia precedes the movement disorder in DLB but not PDD, and is also likely to be different to that found in PD subjects, hence potentially providing a biomarker for the diagnosis of dementia in PD.

9.3 Strengths and Limitations

The strengths of the prevalence study survey include the large sample size and its clinical relevance as it reported actual diagnostic rates in clinical practice. A potential limitation was the inability to verify the diagnoses as it was not feasible to undertake full clinical examinations as part of this study on 5000 cases. However, cases were reviewed with respect to diagnosis by an expert panel, for the purposes of the subsequent in-depth notes study, and a diagnosis of LBD was validated in each case recruited as such.

The diagnostic and management pathway study used objective information collected from medical notes written contemporaneously unlike previous studies of these pathways which were dependent on participant recollection of events and subject to recall bias. The study is limited however by the small sample size.

The strengths of the inflammation study include the use of multiple methodologies including PET imaging, serum inflammatory profiles and clinical assessments to assess the extent and nature of inflammation in DLB subjects. Potential limitations include the use of PK11195 imaging, which is accepted to have a high signal to noise ratio and also displays non-specific binding (Vivash and OBrien, 2016). It nevertheless provides the best means currently available for assessing *in vivo* central inflammation as other ligands for the peripheral benzodiazepine receptor are affected by polymorphisms in the gene for the receptor (Owen *et al.*, 2012). In addition, this is a cross-sectional study and did not test the evolution of inflammation over time and its impact clinically. The serum biomarker study tested a large number of inflammatory markers but was limited by the small sample size.

9.4 Applications to Current Practice

The study showed clinicians in the region tested have high thresholds for diagnosing DLB, often requiring two core features or more and that they rarely use dopamine uptake

scans. One means of improving diagnosis would be to implement a diagnostic toolkit reminding clinicians of the diagnosis criteria and perhaps also the usefulness of dopamine uptake scans. Similarly, an assessment toolkit may help clinicians regularly reviewing PD patients to be aware of the onset of dementia. In addition, this study showed neurologists and geriatricians often referred PD patients to psychiatrists to make a formal diagnosis of dementia. Hence, neurologists specialising in movement disorders and geriatricians regularly seeing PD patients, may benefit from increased education regarding cognition and dementia, to increase their confidence in making a dementia diagnosis themselves. Following this study, the Diamond Lewy study team have indeed introduced an assessment toolkit for the assessment of LBD. We have also carried out teaching sessions for clinicians involved in the study, in the diagnosis of both conditions, with the aim of improving diagnosis of both DLB and PDD.

The study also identified PK11195 binding in the caudate as a potential marker of disease severity in DLB. Hence this biomarker could be used as a prognostic marker clinically or as a marker of treatment efficacy in clinical trials. However this is limited by the short half-life of this ligand of approximately 20 minutes – it is a ^{11}C compound which decays relatively quickly. This means only hospitals with an onsite cyclotron that can generate PK11195 locally would be able to use this ligand, greatly limiting its application. However, if the difficulties associated with the second generation peripheral benzodiazepine receptor compounds (namely how to interpret receptor phenotype) can be overcome, the ligands which are coupled with ^{18}F and therefore have a much longer half-life (of approximately 110 minutes), could be used for these purposes in a much larger number of hospitals.

9.5 Future Studies

The next step, and also a key step to validate the results of this cross-sectional study, is to undertake a longitudinal study with a large number of (50-60) DLB patients. This ideally would be in patients with prodromal DLB (who have core features and cognitive impairment, but who do not have dementia) (Donaghy, O'Brien and Thomas, 2015). An initial PK11195 scan, MRI head scan, peripheral blood cytokine analysis, cognitive and clinical testing as carried out in this study, but also cerebrospinal cytokine analysis, would be followed by a repeat of these assessments after three years. Additional cytokine analysis in the middle of the intervening period could also be carried out. The longitudinal

PET scans would show the trajectory of microglial activation over time and also enable an assessment of their impact on clinical markers plus structural imaging of cortical thickness. If microglial activation declined in subjects as they progressed from prodromal to established disease but remained constant in those that did not decline, this would be a good indication that inflammation has a protective effect. If however microglial activation increased between prodromal and established dementia, with a concurrent decline in clinical and structural markers, this would suggest that inflammation is detrimental. The association with peripheral and cerebrospinal cytokines may reveal the mechanisms behind either effect.

There is also a need to investigate the potentially divergent pathways of microglial activation in AD and DLB. Similar studies as described above but involving AD and DLB subjects are needed to address whether evolution of central inflammation, and its correlation with clinical features, is comparable in AD. This will be of crucial importance to potential immune modulatory therapies in both disorders. If microglial activation is purely beneficial in Lewy body pathology for example, the use of immune suppression therapies in patients with AD with concomitant Lewy body pathology, or vice versa, would be ineffective at best and catastrophic at worst.

Larger DLB and AD cohorts should also be assessed for a broad panel of serum inflammatory markers to try and distinguish the peripheral inflammatory profiles of these disorders and establish a substrate for the use of machine learning tools to enable distinction in dementia subjects once it is clear that a patient indeed has dementia. To this extent the pooling of resources from centres will be vital to obtain sufficient numbers, as DLB subjects are, due to the difficulties in diagnosis already stated, hard to find. This could provide a simple bed-side test to differentiate the two conditions.

Further studies of peripheral inflammation are also required in PDD, in comparison to PD subjects as well as controls and could similarly provide an inflammatory biomarker for the detection of dementia in PD. Comparison with DLB, in terms of the nature and extent of central and peripheral inflammation, is also of importance to understand if similar therapeutic strategies can be used in both.

To study the role of the acquired immune system in Lewy body dementia and also the role of macrophage inflammatory protein-3, interleukin-17A and interleukin-2, flow cytometry to assess peripheral lymphocyte subsets (as has been carried out in PD (Bas *et al.*, 2001), and their relationship to the levels of these three markers, and their

trajectory in relation to clinical features, should be carried out and may confirm a pathological role for CD4 T-cells in Lewy body disorders.

9.6 Conclusion

This study aimed to investigate the rate of Lewy body dementia diagnoses clinically and identify if central and peripheral inflammation could provide a biomarker or source for disease modifying therapy.

A survey of a large number of cases, showed a low rate of LBD diagnosis clinically, with an in depth notes study of a small sample revealing difficulty in the diagnosis of DLB and a delay in the diagnosis of PDD. This study also revealed peripheral inflammation in DLB patients as well as an association between microglial activation and mild disease, consistent with early central inflammation. This suggests any trials on anti-inflammatory approaches in DLB should focus on mild disease.

Further studies are required to distinguish inflammatory pathways in DLB and AD, as well as PDD and PD, to understand the relevant contributions of inflammation between these conditions and to identify accessible biomarkers that will assist in the detection of LBD.

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Appendices

Appendix 1

Published Paper: Clinical Prevalence of Lewy Body Dementia

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Clinical prevalence of Lewy body dementia

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Abstract

Background: The prevalence of dementia with Lewy bodies (DLB) and dementia in Parkinson's disease (PDD) in routine clinical practice is unclear. Prevalence rates observed in clinical and population-based cohorts and neuropathological studies vary greatly. Small sample sizes and methodological factors in these studies limit generalisability to clinical practice.

Methods: We investigated prevalence in a case series across nine secondary care services over an 18-month period, to determine how commonly DLB and PDD cases are diagnosed and reviewed within two regions of the UK.

Results: Patients with DLB comprised 4.6% (95% CI 4.0–5.2%) of all dementia cases. DLB was represented in a significantly higher proportion of dementia cases in services in the North East (5.6%) than those in East Anglia (3.3%; $\chi^2 = 13.6$, $p < 0.01$). DLB prevalence in individual services ranged from 2.4 to 5.9%. PDD comprised 9.7% (95% CI 8.3–11.1%) of Parkinson's disease cases. No significant variation in PDD prevalence was observed between regions or between services.

Conclusions: We found that the frequency of clinical diagnosis of DLB varied between geographical regions in the UK, and that the prevalence of both DLB and PDD was much lower than would be expected in this case series, suggesting considerable under-diagnosis of both disorders. The significant variation in DLB diagnostic rates between these two regions may reflect true differences in disease prevalence, but more likely differences in diagnostic practice. The systematic introduction of more standardised diagnostic practice could improve the rates of diagnosis of both conditions.

Keywords: Dementia with Lewy bodies, Dementia in Parkinson's disease, Epidemiology, Prevalence

Background

Dementia with Lewy bodies (DLB) is a common cause of dementia in older people, characterised by a tetrad of visual hallucinations, fluctuations in cognition, spontaneous parkinsonism, and REM sleep behaviour disorder. Parkinson's disease dementia (PDD) describes dementia arising in the context of established idiopathic Parkinson's disease (PD), and shares both neurobiological and clinical characteristics with DLB. Together, DLB and PDD comprise Lewy body dementia (LBD), conceptualised as a spectrum disorder associated with cortical and subcortical Lewy body pathology, with variations in the temporal onset of motor and cognitive symptoms [1–3].

Validated diagnostic criteria [2] and clinical biomarkers exist for DLB [4, 5]. However, despite the important implications of diagnosis for treatment, mortality [6], and carer well-being [7], previous studies have suggested that only one in three cases is correctly identified in routine clinical care [8, 9] and a considerable lack of consensus surrounds the actual prevalence of DLB.

A recent meta-analysis of epidemiological studies reported that DLB represented 7.5% of all dementia cases in clinical populations [10]. These populations refer to research cohorts in which consecutive referrals to a service or healthcare organisation were screened for DLB on the basis of clinical symptoms and

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investigations. The same meta-analysis found that DLB comprised 4.2% of community-based dementia populations. However, studies contributing to this meta-analysis observed prevalence rates ranging from 0 to 26% in individual cohorts [11, 12].

Variation between individual studies' prevalence rates could represent true differences in DLB prevalence among different regions or countries. However, the wide range of methodological and sampling practices adopted in these studies is an alternative cause for the reported rates.

There is a greater consensus regarding the prevalence of PDD. A systematic review in 2005 found the point prevalence of dementia in PD to be 24.5% [13]. Subsequent studies have reported similar figures of 20–30% [14–16]. Despite the wide variation in the methodology used, the consistency of the rate found suggests it is close to the true proportion of dementia in PD. The systematic review found the prevalence of PDD as a percentage of all dementia cases to be 3.6% [13]. The lifetime prevalence of dementia in PD has also been studied, with 83% of PD patients surviving 20 years developing dementia [17], suggesting that dementia will eventually affect the vast majority of PD patients.

Neuropathological studies report that DLB comprises up to 15–20% of cases of dementia [17, 18], although such cohorts are invariably subject to small sample sizes and selection bias [19, 20]. Furthermore, concomitant Alzheimer's disease (AD) and DLB pathology of varying severity has been found in post-mortem dementia cases, with no clear correlation as yet found with clinical phenotypes of AD or DLB [21]. In addition, many studies fail to correlate clinical data with pathological findings, describing DLB or PDD cases together under the category of LBD. Nevertheless, the 15–20% described in such studies is higher than the reported combined prevalence of DLB (4.2%) and PDD (3.6%) found clinically.

The clinical prevalence of DLB and PDD therefore remains unclear. We aimed to investigate the prevalence in a case series of DLB and dementia in PD across two distinct geographical sites. By employing an identical methodology in two comparable populations, we aimed to identify the rate of diagnosis of these dementias by clinicians in routine practice and better understand the variation in reported LBD diagnosis rates.

Methods

We investigated prevalence in a case series to determine the clinical prevalence of DLB and PDD.

For assessing DLB, nine participating psychiatry of old age/memory clinic services in the UK were identified across four NHS hospital trusts, spread across two distinct geographical areas: East Anglia (EA, n = 2 trusts) and North-East England (NE, n = 2 trusts). Services were chosen by the research team in order to compile a cohort generalisable to that seen in routine clinical practice and included those serving both urban populations and mixed urban and rural populations. Among these were multidisciplinary teams serving urban areas (n = 2), serving rural areas (n = 1), and serving a mixture of both urban and rural populations (n = 6). One service was a tertiary memory clinic combining psychiatry and neurology expertise, and another incorporated a tertiary DLB clinic within a larger secondary care resource. All other services (n = 7) were secondary care organisations. Two clinics were closely affiliated with large teaching hospitals, the remaining seven with smaller district hospitals or community teams. For PDD, five PD or movement disorder clinics, each from a separate NHS trust (EA, n = 3 trusts; NE, n = 2 trusts) were sampled. These consisted of two geriatric medicine services and three which combined geriatric medicine and neurology expertise, serving urban (n = 2) and mixed urban and rural (n = 3) populations. None of these services incorporated specialist tertiary clinics.

The research team reviewed the notes of all subjects seen in services to identify patients with a diagnosis of dementia (for DLB prevalence), and those with a diagnosis of PD (for PDD prevalence), over a fixed 18-month period within a 2-year window from January 2013 to December 2014. Clinical diagnosis, as documented by the practitioner reviewing each patient within respective services, was recorded for each subject, as were age, gender, cognitive score, and date of diagnosis. For the DLB/dementia part of the study, dementia subtype, as determined by the clinician, was recorded. For the PDD/PD part of the study, the dates of diagnosis of both PDD (where applicable) and PD were recorded. Cases were coded as incident (dementia first diagnosed within the 18-month study period) or prevalent (dementia diagnosed prior to the study period, but the subject attended the service during the 18-month window). Patients who attended more than one participating service were included only in the service in which they were first seen. Permission was granted by the UK Confidentiality Advisory Group to collect these limited data from the clinical notes of all patients attending these services without the requirement of informed consent. Ethical approval for the study was also awarded by an NHS Regional Ethics Committee.

Statistical analysis was performed using SPSS 24.0 for Windows. Confidence intervals for prevalence in a case series were calculated using the Wilson method. Mean values and proportions were analysed using

Student's t test for independent samples and the χ^2 test respectively. The Mantel–Haenszel χ^2 test was used to test for a relationship between stratified age group and DLB prevalence. Non-parametric Spearman's rank correlation was used to test for the correlation between the age at PD and the time to the onset of dementia, as the latter showed a non-normal positively skewed distribution. For each test statistic, $p < 0.05$ was regarded as statistically significant.

DLB prevalence in this case series was calculated as the percentage of DLB cases amongst the total number of dementia cases identified. PDD prevalence in the case series was calculated as the number of PD cases diagnosed with dementia, divided by the entire PD population seen during the screening period.

We approached a subset of patients with DLB and PDD, as well as cases matched by age (< 3 years) and gender to patients with non-DLB and PD diagnoses respectively, for consent to access their clinical notes in greater detail. DLB and non-DLB dementia cases were also matched by MMSE score (< 5 points). A panel of three expert clinicians reviewed clinical documentation and applied consensus criteria to each case. This method represents the accepted gold standard to *post-mortem* diagnosis, and has been validated against autopsy and imaging measures [22].

Results

DLB in psychiatry of old age services

The research team reviewed the case notes of 9449 individual patients, of whom 4504 (47.6%) had a dementia diagnosis (Fig. 1, Table 1), other diagnoses being mainly functional psychiatric disorders (such as depression) or cognitive problems falling short of dementia (such as mild cognitive impairment). Patients with DLB comprised 4.6% (95% CI 4.0–5.2%) of all dementia cases. Prevalence in individual services ranged from 2.4 to 5.9%, and was significantly higher among NE services (5.6%; 95% CI 4.8–6.5%; 70% greater) than in EA services (3.3%; 95% CI 2.6–4.2%; $\chi^2 = 13.6$, $p < 0.01$). No significant variation in prevalence was observed within each region (NE, $\chi^2 = 2.54$, $p = 0.28$; EA, $\chi^2 = 4.88$, $p = 0.28$).

Incident DLB cases made up 4.8% (95% CI 4.0–5.7) of dementia cases diagnosed within our study window, ranging from 2.7 to 6.4%. Incidence was also higher in NE services than in EA services (5.8 vs 3.8; $\chi^2 = 5.9$, $p < 0.02$; 53% greater).

DLB prevalence was higher in men ($\chi^2 = 24.8$, $p < 0.01$) (Table 2). In addition, patients with DLB were significantly younger than their non-DLB counterparts (81.2 vs 82.4; $t(4\ 502) = -2.1$, $p = 0.04$), although the mean difference was just over a year, and this age difference was not seen in newly diagnosed cases. DLB prevalence in the case series also negatively correlated with stratified age (Mantel–Haenszel $\chi^2 = 8.2$, $p < 0.01$) (Fig. 2), with similar findings for incident cases (Table 2) indicating that DLB was less commonly diagnosed in older people.

Seventy-five (75/207; 36.2%) DLB cases within the case series consented to a more detailed review of clinical documentation. The diagnosis made in clinical services concurred with that reported by expert clinician panel in 99% of cases (74/75). Expert panel also agreed with clinical diagnosis in 97% (72/74) of cases with non-DLB dementia.

PDD in geriatric medicine and neurology services

The case notes of 2263 individual patients were examined, of whom 1563 (69.1%) had an idiopathic Parkinson's disease diagnosis. PDD comprised 9.7% ($n = 151$, 95% CI 8.3–11.1%) of these PD cases. No significant variation was observed between regions: 8.3% in EA and 10.5% in NE ($\chi^2 = 1.95$, $p = 0.16$). There was also no significant variation found between all services, with PDD prevalence ranging from 4.5 to 11.0% ($\chi^2 = 5.99$, $p = 0.20$).

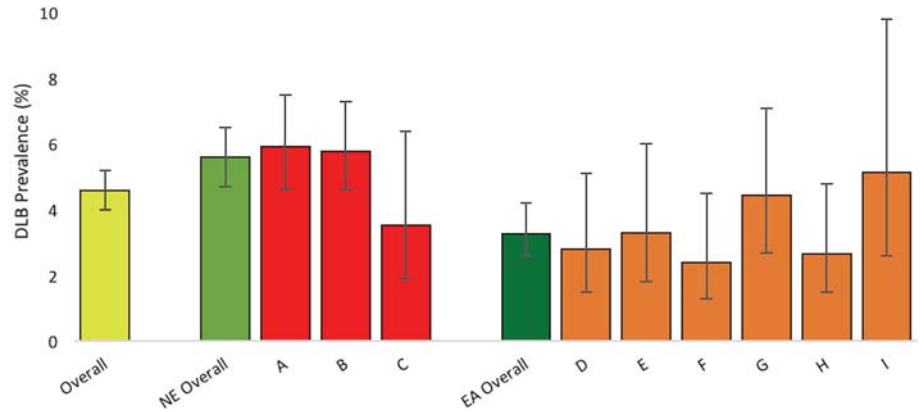

Fig. 1 DLB prevalence by region and service. DLB dementia with Lewy bodies, EA East Anglia, NE North-East England, A–I services

Table 1 DLB prevalence and incidence by region and service

Service	Dementia (all subtypes)		DLB			
	Prevalent	Incident	Prevalent	% of prevalent dementia cases (95% CI)	Incident	% of incident dementia cases (95% CI)
A	1115	548	66	5.9 (4.7–7.5)	35	6.4 (4.6–8.8)
B	1178	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)
North-East England	2575	1291	144	5.6 (4.8–6.5)	75	5.8 (4.7–7.2)
D	355	204	10	2.8 (1.5–5.1)	9	4.4 (2.3–8.2)
E	302	169	10	3.3 (1.8–6.0)	7	4.1 (2.0–8.3)
F	377	186	9	2.4 (1.3–4.5)	5	2.7 (1.2–6.1)
G	361	212	16	4.4 (2.7–7.1)	10	4.7 (2.6–8.5)
H	378	357	10	2.7 (1.4–4.8)	10	2.8 (1.5–5.1)
I	156	150	8	5.1 (2.6–9.8)	7	4.7 (2.3–9.3)
East Anglia	1929	1278	63	3.3 (2.6–4.2)	48	3.8 (2.8–4.9)
Overall	4504	2569	207	4.6 (4.0–5.2)	123	4.8 (4.0–5.7)

CI confidence interval, *DLB* dementia with Lewy bodies

There was a male predominance in PD cases but no significant differences in gender found when comparing the two regions, in those with PDD, or when considering the larger cohorts of all PD patients (including PDD) between the regions (Table 3).

However, both PD and PDD subjects were older in EA than in NE (PD mean difference of 2.8 years, $p < 0.001$; PDD mean difference of 2.7 years, $p = 0.03$).

Significantly more incident cases of PDD (newly diagnosed within our screening period) were found within EA compared to NE, comprising 59.1% of all PD cases in EA compared to 40.0% of cases in NE ($\chi^2 = 4.49$, $p = 0.034$; Fig. 3). In addition, significantly lower Mini-Mental State Examination (MMSE) scores at the time of PDD diagnosis were recorded in EA than in NE (Mann–Whitney *U*, $p = 0.008$; Fig. 4).

A highly significant inverse correlation between age at initial PD diagnosis and time until dementia onset (Spearman's correlation, $\rho = -0.66$, $p < 0.001$) was also found in the PDD group as a whole (Fig. 5).

Table 2 Age and gender of DLB and non-DLB patients

	DLB	Non-DLB	*p*
Age at screening (± SD)			
Prevalent	81.3 (± 7.8)	82.4 (± 7.8)	0.04
Incident	81.8 (± 7.6)	82.1 (± 8.1)	0.59
Gender, male/female (% male)			
Prevalent	113/94 (54.6%)	1607/2690 (37.4%)	< 0.01
Incident	62/61 (50.4%)	958/1488 (39.2%)	0.01

DLB dementia with Lewy bodies, *SD* standard deviation

The diagnosis of the expert panel concurred with the diagnosis documented in the clinical notes in 97% of PDD cases consented for detailed notes review (37/38) and in 100% of recruited PD cases (35/35).

Discussion

We found that DLB comprised 4.2% of all dementia cases in a representative clinical population in NHS secondary care services. This is a considerably lower figure than that cited by both neuropathological studies and previous meta-analyses [10, 18]. We also found dementia diagnosed in only 9.7% of cases of PD, much lower than the 20–30% seen in the systematic review [13] and subsequent population and clinic-based studies of PDD prevalence [14–16].

Our study was deliberately designed to determine the frequency of diagnoses in routine clinical services, and reflects current real-life practice for patients being assessed in specialist services within secondary care. Services were selected by the research team primarily on the basis of their generalisability to psychiatry of old age and neurology/geriatric medicine services, throughout the UK.

The most likely reason that rates found in our cohorts are lower than those reported in meta-analysis of other hospital-referred populations, and indeed nearer to community-based estimates, is probably to be found in the methodology employed. Our study was based upon scrutiny of routine clinical records from services receiving mainly community-based referrals. This cohort therefore represents a broader, more generalisable dementia population than those investigated in prevalence

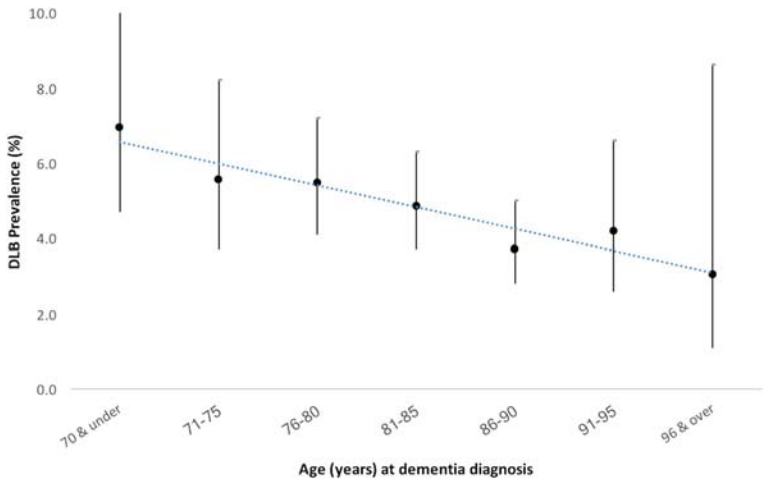

Fig. 2 DLB prevalence and age at dementia diagnosis. DLB dementia with Lewy bodies

studies conducted within specialist centres that often show larger prevalence rates.

Nevertheless, our observed range in prevalence in a case series likely also reflects a lower rate of disease detection, rather than true disease prevalence in some populations. This is supported by the differences in prevalence of DLB observed between our NE and EA cohorts, and the wide range in rates observed in neighbouring services within the same region. This variation in detection may be related to a number of factors; the effect on medical education, training, and service development of Newcastle University's long history of LBD research may have contributed to higher rates in NE. Varying sensitivity to core DLB features may play a role in detection; Walker *et al.* [23] noted that prevalence studies incorporating a neurological examination reported higher prevalence rates of DLB. It is also possible that not all practitioners comprising participating services are fully aware of consensus criteria, but the high level of agreement between diagnoses made within services and those made by the expert panel (98%) would suggest that consensus diagnostic guidelines are in routine use in participating services.

Despite our belief that our findings represent variation in DLB detection, variation in true disease prevalence cannot be entirely ruled out. Environmental factors or a combination of environmental factors in the pathogenesis of DLB have been proposed [24]. It is not possible to discount the possibility that the variation in regional diagnostic rates seen within this study simply reflect the degree of exposure to causative or precipitating biological factors, but the intra-regional variation which was also seen would argue against this.

Contrary to the findings of the meta-analysis, which reported a positive relationship between age and DLB prevalence (although this was not statistically significant), we identified an inverse correlation between these two factors, and found the mean age of DLB patients at diagnosis to be lower than that of non-DLB dementia patients. This may be a reflection of a more aggressive course and increased mortality in DLB, or that DLB becomes less common clinically with advancing age as other pathologies become more prevalent leading to a mixed pathological and clinical picture. Our study design and information systems did not allow us access to accurate mortality

Table 3 Group demographics and differences between regions

Demographics	North-East England	East Anglia	Group difference
Gender (PDD), males/females	78/23	35/14	$\chi^2 = 6.0$, $p = 0.44$
Gender (all PD), males/females	587/385	328/260	$\chi^2 = 3.2$, $p = 0.07$
Age (years) at PDD onset, mean (± SD)	75.6 (± 6.7)	78.3 (± 7.3)	$t = -2.1$, $p = 0.03$
Age (years) at PD onset, mean (± SD)	70.3 (± 9.7)	73.1 (± 8.6)	$t = 5.8$, $p < 0.01$
Age at midpoint of screening period (all PD), mean (± SD)	76.9 (± 7.2)	78.7 (± 6.9)	$t = 4.7$, $p < 0.01$

PD Parkinson's disease, *PDD* Parkinson's disease dementia, *SD* standard deviation

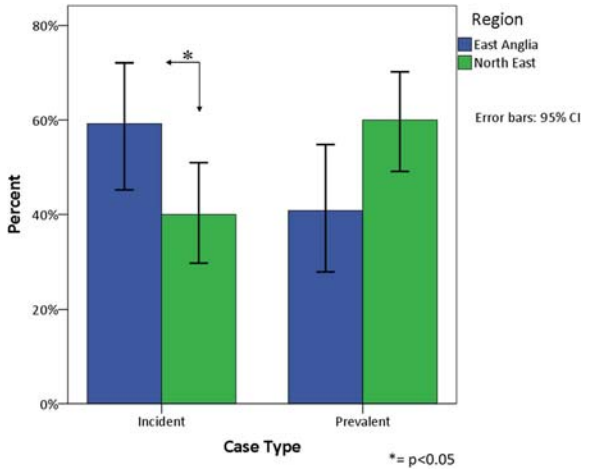

Fig. 3 Percentage of cases of PDD diagnosed within our screening period compared to cases diagnosed before our screening period. Confidence interval (CI) calculated using standard (approximate method)

data, although increased mortality in DLB has been described [6].

DLB was also more prevalent among men than women in our cohort, a finding which also conflicts with the lack of significant association identified in meta-analysis [10]. A male preponderance has been observed in neuropathological DLB samples [25] but population samples have both supported and refuted this hypothesis [26, 27]. Our very large sample size and multi-servicing sampling make our data the strongest support for a male preponderance of DLB from clinical samples to date.

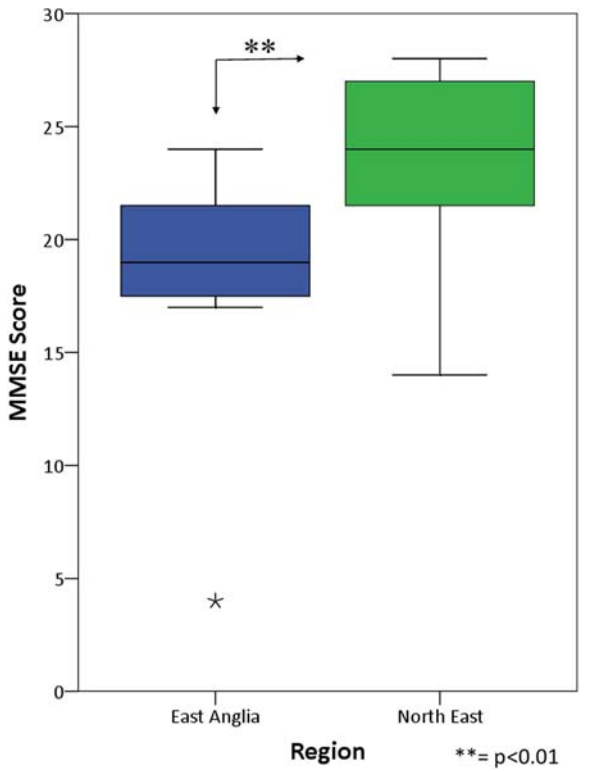

Fig. 4 Comparison of cognitive scores at the time of PDD diagnosis between regions. MMSE Mini-Mental State Examination

Dementia prevalence in our PD cohorts was much lower than has been reported previously. A variation in prevalence of dementia was not identified between regions, yet higher age and lower MMSE scores at diagnosis of dementia suggest that dementia is diagnosed later on in the disease in EA. However, as the age at PD diagnosis was also older in EA, once again the possibility that there may be an environmental factor driving earlier onset in NE cannot be discounted. Another reason behind the difference in age may be the differences in life expectancy between the regions – the latest figures show this to be 80.4/83.8 years (male/female) in EA and 78.0/81.7 years in NE [28] – similar to the age differences we observed between the two regions in the study. It is, however, possible that clinicians in the NE region have a lower threshold for making both diagnoses. It should also be noted that the mean age at the mid-point of our screening period across both regions was 77.6 years and was similar to the median of the mean ages in studies analysed in the systematic review by Aarsland *et al.* (74.9 years) [13].

The strong inverse correlation between age at onset of PD and the time to diagnosis of dementia is consistent with age being a risk factor for PDD [29].

As with DLB, the most likely cause of the lower prevalence rate of PDD in our case series is because we have reported the observed rate of diagnosis of PDD as made by clinicians in routine practice. Previous studies have sought to identify dementia specifically in their PD populations using standardised diagnostic tools. Although clinical diagnoses agreed with those made by our independent clinician panel in 99% of PDD and PD cases, it is likely that our findings reflect lower detection rates of PDD within the PD population.

A lower rate of diagnosis in clinical practice has important implications for the patients and their carers who benefit from a diagnosis being made. The development of dementia has a profound effect on the patient and carer, and allows for the provision of support services to cater for these. Dementia leads to loss of insight, poor judgement, poor financial decision-making, increased carer stress, impaired driving skills, and an increased falls risk, amongst other difficulties [17]. Healthcare providers would also need to adapt their services to cater for a higher population of their patients experiencing the difficulties of having dementia.

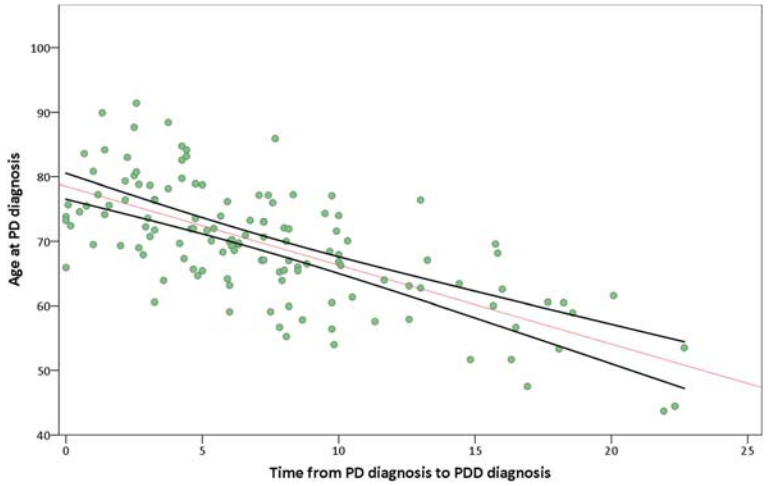

Fig. 5 Negative correlation between age at initial diagnosis of PD and time before dementia onset. PD Parkinson's disease, PDD Parkinson's disease dementia

Strengths of this study include the very large sample size compared to previous studies, its multi-site nature (when previous estimates have usually involved only single sites), its representativeness, in that access to all cases within a service was allowed, and, since we used clinically made diagnoses, its clinical relevance. Potential limitations include the fact that we could not compare diagnostic rates made by clinicians with "true" prevalence, which would have required full clinical examination of all 12,500 cases and would not have been possible. Another important limitation of the study is that our methodology permitted investigation of DLB and PDD prevalence as determined by primary clinical dementia syndrome alone. We were therefore unable to determine the contribution of co-existing AD neuropathology in such cases, although no mechanism currently exists to accurately determine such cases on the basis of clinical presentation [21].

Conclusion

Our study identified clinical prevalence rates of DLB and PDD in a case series considerably lower than that reported by clinical epidemiological cohorts and neuropathological studies. Importantly, we observed significant differences in the rates of DLB diagnosis among different regions, and a preponderance of DLB among males and younger patients. We found no such regional variations in prevalence amongst our clinical PDD population, but did find that PDD cases in EA were older, with a lower MMSE score, at the point of dementia diagnosis. Although our observation of regional variation in diagnosis could be attributed to different patterns of disease prevalence, a more likely explanation is that varying clinical diagnostic practices produce differences in DLB and PDD detection, rather than true disease prevalence.

Since it is important to accurately recognise and diagnose both DLB and PDD to optimise clinical care and management, and service delivery, and to allow more accurate prognosis, methods by which diagnostic rates might be improved should be tested. This might include the introduction of standardised assessments and scales to facilitate accurate recognition of DLB and PDD, including widespread use of the new DLB criteria [3], instruments such as the Lewy body composite risk score [30], or the DLB/PDD diagnostic toolkits [31].

Abbreviations

AD: Alzheimer's disease; DLB: Dementia with Lewy bodies; EA: East Anglia; LBD: Lewy body dementia; MMSE: Mini-Mental State Examination; NE: North-East England; NHS: National Health Service (UK); PD: Parkinson's disease; PDD: Parkinson's disease dementia; UK: United Kingdom

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request after completion of the DIAMOND-Lewy programme.

Authors' contributions
JPMK and AS contributed equally to the article, leading data analysis and writing of the manuscript. JPMK, AS, AB, and SAHB conducted the data collection process. JTO and DJB contributed equally to the study approval and funding process, and supervised data collection and analysis at their respective sites. All authors contributed to the design of this study and read, contributed to, and approved the final manuscript.

Ethics approval and consent to participate
Permission was granted by the UK Confidentiality Advisory Group to collect limited data from the clinical notes of all patients attending participating services without the requirement of informed consent. Ethical approval for the study was also awarded by an NHS Regional Ethics Committee (NRES Committee North East—Newcastle & North Tyneside 1; Reference 13/NE/0268).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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Appendix 2

Published Paper: Neuroinflammation in Lewy Body Dementia

Parkinsonism and Related Disorders 21 (2015) 1398–1406

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Editor's Comment: The potential role of neuroinflammation in neurodegenerative disease processes has received increasing attention in recent years. In this timely review, Surendranathan and colleagues provide a masterful summary of what is known about neuroinflammation in Parkinson's disease dementia and dementia with Lewy bodies. They shepherd us through the bewildering array of factors promoting or inhibiting microglial activation in these two disorders and succinctly review the neuroimaging, neurochemical, neuropathological, genetic and epidemiologic evidence for neuroinflammation in these Lewy body disorders.

Ronald F. Pfeiffer, Editor-in-Chief, Dept. of Neurology, Oregon Health and Science University (OHSU), 3181 SW Sam Jackson Park Rd, Portland, OR 97201-3098, Oregon, USA.

Review article

Neuroinflammation in Lewy body dementia

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ABSTRACT

Neuroinflammation is increasingly recognized as a key factor in the pathogenesis of neurodegenerative conditions. However, it remains unclear whether it has a protective or damaging role. Studies of Alzheimer's disease and Parkinson's disease have provided much of the evidence for inflammatory pathology in neurodegeneration. Here we review the evidence for inflammation in dementia with Lewy bodies and Parkinson's disease dementia.

Neuroinflammation has been confirmed *in vivo* using PET imaging, with microglial activation seen in Parkinson's disease dementia and recently in dementia with Lewy bodies. In Parkinson's disease and Parkinson's disease dementia, microglial activation suggests a chronic inflammatory process, although there is also evidence of its association with cognitive ability and neuronal function.

Alpha-synuclein in various conformations has also been linked to activation of microglia, with a broad range of components of the innate and adaptive immune systems associated with this interaction.

Evidence of neuroinflammation in Lewy body dementia is further supported by pathological and biomarker studies. Genetic and epidemiological studies support a role for inflammation in Parkinson's disease, but have yet to provide the same for Lewy body dementia.

This review highlights the need to identify whether the nature and extent of microglial activation in Lewy body dementia can be linked to structural change, progression of domain specific cognitive symptoms and peripheral inflammation as a marker of central microglial pathology. Answers to these questions will enable the evaluation of immunotherapies as potential therapeutic options for prevention or treatment of dementia with Lewy bodies and Parkinson's disease dementia.

1. Introduction

Lewy body dementias (LBDs) include the closely related conditions of dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD). The clinical syndrome of DLB forms at least 4.2% of all dementia patients and is second only to Alzheimer's disease (AD) as a cause of degenerative dementia in older people [1]. Dementia also develops in over 80% of those with Parkinson's disease (PD) [2], a disorder where Lewy bodies play a prominent role, with PDD forming 3.6% of all dementia cases [3]. Autopsy studies of dementia cases have estimated the combined prevalence rate of LBDs to be even higher, at around 20% [4,5].

The etiology of LBDs is unclear, but a role for chronic neuroinflammation has been proposed, analogous to the emerging

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evidence for inflammation in the etiology of AD. The evidence to date for AD includes neuropathological studies with evidence of brain inflammation, Positron Emission Tomography (PET) imaging displaying microglial activation *in vivo*, genetic studies implicating polymorphisms in genes involved in the inflammatory response as risk factors, epidemiological studies indicating a protective effect of non-steroidal anti-inflammatory drugs (NSAIDs) and mouse models of AD in which NSAIDs reduced neuroinflammation and protein deposition [6–9].

In light of the gathering evidence for neuroinflammation in AD, we asked whether neuroinflammation is also involved in the etiology of LBDs. We review the literature for evidence of neuroinflammation in Parkinson's disease dementia and dementia with Lewy bodies, across multiple methodologies.

2. Literature search strategy

References were identified using searches of PubMed with key words. The following combinations were used in a search of titles and abstracts in June 2015 (the number of articles yielded is noted in brackets):

1. 'Lewy' and ('inflammation' OR 'neuroinflammation') (98 articles)
2. ('Parkinson's disease dementia' OR 'PDD' OR 'DLB' OR ('Dementia AND Parkinson*')) AND ('neuroinflammation' OR 'inflammation') (283 articles)
3. 'synuclein' AND 'microglia' (185 articles)
4. 'synuclein' AND ('inflammation' OR 'neuroinflammation') (210 articles)

The abstracts of these articles were screened and full texts of those potentially relevant articles to the review were obtained. In order to ensure that all relevant references were sourced, references were in turn reviewed for other relevant articles, supplemented by articles known to the authors.

3. Microglial function

Neuroinflammation describes the response to injury within the central nervous system (CNS) leading to the activation of microglia and astrocytes, release of cytokines and chemokines, invasion of circulating immune cells and complement activation. Microglia are the resident macrophages of the CNS, originating from progenitors in the embryonic yolk sac [10]. They provide the innate immune response to invading pathogens and also initiate the adaptive response through antigen presentation [11].

Microglia are resting or "inactivated" under physiological conditions with characteristic ramified morphology and distributed within brain regions, such that rami are close but not touching, implying each cell has its own distinctive territory. But even in this inactive state, they have been shown using two-photon microscopy to be continuously monitoring the extracellular spaces with their processes and protrusions in adult mice [12]. Activation leads to morphological change to a more rounded amoeboid shape, with targeted movement of processes towards sites of injury or stimuli to initiate phagocytosis [12] and leads to production of chemokines, that amplify the response by recruiting other microglia, plus cytokines, free radicals and proteases which destroy infectious organisms and infected neurons.

Microglia appear to have an important part both in MPTP disease progression and idiopathic PD [13], suggesting a central role for these glia in nigro-striatal degeneration, irrespective of etiology. Microglia may be especially susceptible to mechanisms of aging. Their maintenance is proposed to be dependent on self-renewal rather than replenishment by peripheral blood precursors [14,15], which could be highly significant in age dependent neurodegenerative conditions such as LBD. Systemic infections or disease, which rise in number with age, could also lead to priming of microglia, such that their response is exaggerated and damaging to nearby neurons leading to cognitive decline [16]. It has also been proposed that an initial stimulus that triggers microglial activation could persist in neurodegenerative disorders leading to repeated cyclical chronic neuroinflammation causing neuronal dysfunction and cell death [17,18]. The specificity of these changes to Lewy body dementias is unclear.

4. Imaging evidence of neuroinflammation and neuronal dysfunction

Imaging studies have shown an association between neuroinflammation *in vivo* and cognitive dysfunction. Microglial activation as a marker of neuroinflammation has been identified in PD and PDD [19] (see Table 1) using [^{11}C]-*R*PK11195 (RPK11195), a PET ligand that binds to a translocator protein found on microglia in their activated state. Extensive microglial activation has similarly been identified in another α-synucleinopathy: multiple systems atrophy [20], as well as other degenerative conditions, including AD [21,22].

An association between microglial activation in the midbrain and dopaminergic loss in the dorsal putamen has been found in the early stages of PD (less than 2.5 years), both contralateral to the clinically affected side, with levels of activation correlating with severity of motor impairment measured by the Unified Parkinson's Disease Rating Scale (UPDRS) [23]. In the later stages of disease (disease duration range 0.5–21 years), there is extensive microglial activation, with the basal ganglia, cortex and pons all showing significantly increased levels. The substantia nigra was however spared. Follow-up scans in eight of these subjects (after 18–28 months) showed no significant change in microglial activation from baseline despite a clear deterioration in disability as measured using the UPDRS. Cognition was however not assessed longitudinally [24]. The authors also noted a clear overlap in the areas of microglial activation and the regions proposed by Braak et al. [25] in their study of PD pathology. In PDD subjects, there is increased cortical microglial activation compared to control subjects, however levels of activation were also increased in comparison to PD cases – in the left parietal lobe [26].

In DLB, increased microglial activation in the substantia nigra and putamen, plus several cortical regions was found in a pilot imaging study of six cases of less than one year's duration [27]. That microglial activation occurs in more widespread regions in early DLB, where there is greater cognitive dysfunction compared to early PD, strengthens the link between microglial activation and cognitive decline.

A relationship between microglial activation and cognitive function was indeed found in PDD, where cortical activation levels inversely correlated with MMSE in temporo-parietal, occipital, and frontal cortical regions [19,26]. Fan et al. [19] demonstrated a significant negative correlation between whole brain levels of microglial activation and glucose metabolism. Within the temporo-parietal cortex there was voxel by voxel significant inverse correlation between levels of microglial activation and glucose metabolism in the immediate vicinity suggesting local damage, but the areas of correlation were small. The authors however suggest distant microglial activation could be linked to cell dysfunction in the medial temporal lobe through pre-existing neuronal pathways. Neither study of PDD assessed whether areas of increased activation (such as in the hippocampus) were linked to dysfunction in specific cognitive domains (such as memory), which may have

Table 1
Evidence of *in vivo* microglial activation in PD, PDD and DLB from RPK11195 PET imaging studies.

Study	Participant numbers (controls)	Participant age (years)	Participant MMSE	Disease duration (years)	Regions with increased microglial activation compared to controls
Ouchi et al., 2005 [23]	10 PD (10 controls)	Range: 43–72; Mean: 59.6	Range: 26–30; Mean: 28.3	Range: 0.4–2.5; Mean: 1.4	Midbrain contralateral to the clinically affected side
Iannaccone et al., 2013 [27]	6 PD (11 controls)	Range: 60–74; Mean: 70.2	Range: 27–30; Mean: 29	Range: 0.6–1; Mean: 0.8	Putamen, substantia nigra
Gerhard at al., 2006 [24]	18 PD (11 controls)	Range: 50–69; Mean: 59.2	Not specifically stated, screening tests normal in PD group	Range: 0.5–21; Mean: 8.6	Striatum, pallidum, thalamus, cortex (precentral gyrus, frontal lobe, anterior cingulate gyrus, posterior cingulate gyrus) and pons
Edison et al., 2013 [26]	8 PD (10 controls)	Range: 58–75; Mean: 68.2	Range: 27–30; Mean: 28.8	Mean: 9.2	Cortex (temporal, parietal, and occipital regions)
Fan et al., 2014 [19]	11 PDD (8 controls)	Range: 55–75; Mean: 68.4	Mean: 22.1	Not stated	Anterior cingulate gyrus, posterior cingulate gyrus, frontal lobe, temporal lobe, parietal lobe, occipital lobe, medial temporal lobe, amygdala and hippocampus
Edison et al., 2013 [26]	11 PDD (10 controls)	Range: 56–80; Mean: 69.3	Range: 16–26; Mean: 21.8	PD duration mean: 10.6; Dementia duration mean: 3.5	Striatum, cortex (frontal, temporal, parietal, anterior and posterior cingulate gyrus, and occipital cortical regions)
Iannaccone et al., 2013 [27]	6 DLB (11 controls)	Range: 62–82; Mean: 72	Range: 19–30; Mean: 24	Range: 0.7–1; Mean: 0.8	Caudate, putamen, thalamus, substantia nigra, cortex (frontal lateral, parietal lateral, temporal lateral, temporal pole, precuneus, occipital medial, occipital lateral, anterior cingulate, posterior cingulate) and cerebellum

provided a stronger link between inflammation and cognitive dysfunction.

Small clusters of positive correlations were also found between RPK11195 binding and amyloid load (as determined by [^{11}C] Pittsburgh compound B (PIB), a marker of fibrillary amyloid load) in PDD subjects, but only in the parietal lobe and anterior cingulate, as opposed to AD subjects in whom there was a stronger correlation between amyloid load and microglial activation. There was however little amyloid deposition found in PDD cases overall [19]. Proteins other than amyloid, such as α-synuclein or tau, could be triggering microglial activation in PDD, however currently there are no α-synuclein PET ligands available to demonstrate this and tau ligands have only very recently become available.

Overall small scale studies with *in vivo* imaging have suggested that in PD, PDD and in a small preliminary report of DLB, there is early microglial activation. But, this does not appear to increase over time. Significantly microglial activation also correlates inversely with cognitive function and to an extent protein deposition, suggesting microglia may have a crucial role in the pathogenesis of these conditions.

5. Alpha synuclein and neuroinflammation

The evidence for extensive microglial activation in LBDs, in an immunologically privileged site such as the brain, is highly significant. Immune responses are tightly controlled and yet there is widespread glial cell activation, present chronically during the disease. The initiation of the innate response occurs through pattern-recognition receptors (PRRs) expressed on CNS cells (for example the toll-like receptor (TLR)) through activation by pathogen associated molecular patterns or danger associated molecular patterns. However α-synuclein is the main component of Lewy bodies [28] which characterize LBDs, and the driving force behind the disease process, hence the interaction between this protein and microglia appears to be critical. Alpha-synuclein inclusions in neurons and glia are associated with DLB and PDD, as well as PD and multiple system atrophy. In DLB and PDD, the inclusions are neuronal and in the form of Lewy bodies [28]. Lewy neurites are also common in these disorders, consisting of coarse dystrophic neurites immunoreactive for α-synuclein within affected neurons. With 140 amino acids, α-synuclein's possible intracellular forms include monomeric [29,30] or relatively stable folded tetramer [31,32].

Alpha-synuclein, has been shown repeatedly to activate microglia and induce dopamine cell loss [33–35], including monomeric wild-type and mutant forms as well as extracellular oligomeric conformations. Indeed, neuron-glia cultures depleted of microglia have been shown to be resistant to α-synuclein induced dopaminergic neurotoxicity [33]. More recently the focus has moved on to possible mechanisms. Models of PD have been used to study this relationship rather than models of DLB, with overexpression of α-synuclein in the substantia nigra using viral vectors, the most common. A survey of the literature shows several possible mechanisms for this interaction (see Table 2).

A number of immunomodulatory proteins and compounds are implicated in α-synuclein microglial recognition, chemotaxis, activation and response. TLRs 1 [36], 2 [36,37] and 4 [38] are PRRs key to the innate response machinery and have been reported as having a role in recognition of α-synuclein by microglia. Microglia exposed to higher-ordered oligomers (but not monomers) of α-synuclein changed to an amoeboid, phagocytic morphology with increased secretion of Tumor Necrosis Factor α (TNF-α) that was reduced by inhibition of the TLR 1/2 complex [36]. A separate study found only β-sheet rich oligomeric conformations of α-synuclein could activate microglia via TLR 2, but both aggregated and non-aggregated forms could activate microglia through TLR 4. Furthermore pro-inflammatory cytokine/chemokine release was completely eliminated in TLR 2 knockout mouse microglia exposed to α-synuclein, but remained unaffected in TLR 4 knockout mouse microglia [39], suggesting TLR 2 recognition of oligomeric α-synuclein leads to inflammation.

Another molecule which could feature in the initiation of microglia activation is Fractalkine, a membrane bound chemokine which acts on the CX3CR1 receptor on microglia to suppress production of inflammatory molecules. A soluble secreted form of Fractalkine had a protective function in an animal model of α-synuclein overexpression, suggesting loss of this membrane bound chemokine could lead to neuronal loss through microglia mediated cell damage [40].

Table 2
Potential mechanisms of interaction between α-synuclein and microglia.

Interaction/receptor	Proposed mechanism of microglial interaction with α-synuclein	PD model	References
TLR 1&2 complex	**Oligomeric α-synuclein induces a pro-inflammatory microglial phenotype through TLR 1/2 complex:** microglia exposed to oligomers of α-synuclein changed to an amoeboid, phagocytic shape, with increased secretion of TNF-α and interleukin-1b. TNF-α secretion was reduced by the addition of a TLR-1/2 complex inhibitor or by a MyD88 inhibitor.	Primary microglia cultures derived from mouse cortices were exposed to high-order oligomeric forms of purified human wild-type α-synuclein	[36]
Fractalkine receptor (FKN), an immune regulatory protein	**Secreted form of FKN is neuro-protective**: Soluble secreted form of FKN prevents reduction in tyrosine hydroxylase cell staining compared to controls and membrane bound FKN models when exposed to overexpression of α-synuclein, despite increased MHCII expression on microglia.	Overexpression of human α-synuclein via viral vector combined with a variety of viral constructs of FKN	[40]
CD11b receptor	**Alpha-synuclein binds to CD11b on microglia to direct microglial migration:** neuronal α-synuclein overexpression led to microglial migration toward neurons, which was reduced by antibodies to the CD11b receptor and diminished in CD11b knockout mice.	Overexpression of human α-synuclein via viral vector in rat primary neuron-enriched cultures	[41]
Galectin-3 (carbohydrate-binding protein and inflammatory mediator)	**Galectin 3 mediates microglial cytokine release**: Release of Interleukin-2 and Interleukin-12 after exposure to monomeric and aggregated forms of recombinant α-synuclein reduced by genetic down regulation or pharmacological inhibition of galectin-3.	Microglia from wild-type and galectin-3 knockout mice	[46]
Leucine-rich repeat kinase 2 (LRRK2)	**LRRK2 required for microglial activation and dopaminergic degeneration**: Rats lacking LRRK2 demonstrated a significant reduction in microglial activation compared to wild type mice rats, when exposed to lipopolysaccharide (LPS) and were protected from dopaminergic neurodegeneration from α-synuclein overexpression.	Rats exposed to intracranial LPS injection or overexpression of human α-synuclein via viral vector	[47]
β1-integrin	**Migration of microglia to disease affected regions is via β1-integrin**: β1-integrin inhibition reduced microglial morphological changes and motility (as shown by reduced wound healing).	Rat primary microglia exposed to α-synuclein conditioned medium (αSCM)	[42]
Interleukin-1 (IL-1)	**IL-1 is required for microglial activation**: behavioral deficiencies that occurred in wild-type mice, following LPS administration did not occur in IL-1 knockout mice. Tyrosine Hydroxylase gene expression was similarly preserved in IL-1 knockout but not wild-type mice.	Mouse model using intracranial LPS injection into wild-type and IL-1 (α and β) knockout mice	[44]
MHCII Complex	**MHCII complex mediates microglial activation and dopaminergic cell loss**: overexpression of synuclein leads to induction of MHCII expression on microglia and genetic knockout of MHCII prevents microglial activation, IgG deposition and dopaminergic cell loss *in vivo*.	Mouse model using overexpression of human α-synuclein via viral vector in wild-type and MHCII knockout mice	[50]
TLR 4	**TLR 4 mediates microglial phagocytic activity and cytokine release in the presence of α-synuclein:** Microglial phagocytic activity was significantly reduced in TLR4 knockout microglia mice after treatment with different forms of α-synuclein; knockout mice also showed significantly reduced TNF- α production following treatment with α-synuclein.	Mouse primary microglia from wild type and TLR4 knockout mice challenged with cloned human α-synuclein from spinal cord cDNA	[38]
TLR 2	**TLR 2 mediates microglial activation by oligomeric α-synuclein**: TLR2 knockout mice exhibited significantly lowered microglial activation compared with wild type mice when exposed to α-synuclein overexpression; cytokine/chemokine gene induction following exposure to αSCM, was prevented by antagonizing TLR2 and by depletion of the TLR2 gene; and TLR2 was only activated by oligomeric alpha synuclein not the dimer or monomer forms.	Mouse model using overexpression of human α-synuclein via viral vector in wild-type and TLR 2 knockout mice; oligomeric human α-synuclein proteins released from dSY5Y cells	[37]

(*continued on next page*)

Table 2 (*continued*)

Interaction/receptor	Proposed mechanism of microglial interaction with α-synuclein	PD model	References
Fc gamma receptor (FcγR)	**FcγR mediates α-synuclein intracellular localization to autophagosomes and NF-κB pro-inflammatory signaling**: microglia internalized α-synuclein in a dense aggregated form in wild-type mice but a diffuse manner in FcγR knockout mice; FcγR knockout mice treated with α-synuclein also failed to trigger the enhancement of nuclear NF-κB p65 seen when wild-type mice are exposed to α-synuclein.	Primary microglial cultures from wild-type and FcγR knockout mice, treated with human α-synuclein	[49]
NRF2 (NF-E2-related factor 2), a transcription factor	**NRF2 protects against α-synuclein mediated microglial activation and dopaminergic cell loss:** NRF2 knockout mice showed increased microglial activation and greater nigral dopaminergic neuronal loss than wild-type mice when exposed to α-synuclein overexpression; NRF2 knockout neurons were characterized by thick dendrites loaded with α-synuclein, similar in appearance to Lewy neurites and this was associated with reduced levels of the beta subunit (PSMB7) of the catalytic core 20S proteasome compared to wild-type mice.	Mouse model using overexpression of human α-synuclein via viral vector in wild-type and NRF2 knockout mice	[48]
Prostaglandin E2 receptor subtype 2 (PGE2)	**PGE2 is key to regulation of aggregated α-synuclein levels:** microglia isolated from PGE2 knockout mice exhibited enhanced clearance of aggregated α-synuclein and showed increased resistance to MPTP with less aggregated α-synuclein in the substantia nigra and striatum.	Aggregated α-synuclein from human DLB cases incubated with wild-type and PGE2 knockout mice microglia	[51]

Alpha-synuclein, in extracellular aggregated form, has been shown to be a chemoattractant through CD11b receptors on microglia [41]. Also, the β1-integrin subunit, which forms transmembrane adhesion molecules has been reported as being required for the morphological changes and migration of microglia seen in the presence of extracellular α-synuclein [42].

Once microglia are activated, Interleukin-1 (IL-1) appears to be a key cytokine in promoting an inflammatory response. IL-1α and β knockout mice did not show loss of dopamine neurons or behavioral deficits seen in wild-type mice in a mouse model of PD, utilizing lipopolysaccharide(LPS) injections into the substantia nigra. LPS injections have been shown to produce microglial activation, cytokine release and subsequent dopaminergic cell loss in the substantia nigra [43]. TNF-α knockout mice however showed similar results to wild-type mice [44], indeed TNF-α may have role in promoting α-synuclein accumulation [45]. Galectin-3 has also been shown to be important for the inflammatory effect of α-synuclein. Its inhibition significantly reduced cytokine release by microglia in response to aggregated α-synuclein [46].

Leucine-rich repeat kinase 2 (LRRK2) is a protein expressed on microglia when they are in their inflammatory state and has been shown to have a significant role in α-synuclein mediated microglial activation and subsequent cell loss, with LRRK2 knockout mice being protected from α-synuclein overexpression [47]. Another protein involved is NRF2, which is a transcription factor for a number of cell protection proteins and appears to have a protective role in the interaction [48].

Several studies suggest the adaptive immune response is engaged by microglia following their activation. Knockout mice without Fc gamma receptors (FcγR), which are found on microglia and involved in facilitating phagocytosis through binding of IgG, showed reduced pro-inflammatory signaling in the presence of aggregated α-synuclein. Suggesting the latter could be triggering inflammation and antibody mediated cell damage through FcγR [49]. In addition, a knockout of all four murine MHC II complex genes prevented α-synuclein induced dopaminergic cell loss in a mouse model, strongly suggesting that CD4 T lymphocytes are critical to α-synuclein cell damage. Microglia, as the only resident cells expressing MHC class II in the CNS, would be candidates for their recruitment, although infiltrating antigen presenting cells such as macrophages may also be involved [50]. Furthermore, mice with microglia deficient in Prostaglandin E2, which is thought to have a role in lymphocyte proliferation, have increased resistance to MPTP mediated pathology [51].

6. Pathological evidence of inflammation

Pathological studies further support a role for inflammation. Large numbers of HLA-DR-positive microglia, indicating reactive states, have been reported in the substantia nigra of PD and PDD cases together with Lewy bodies in association with a reduction in dopaminergic cells. In the PDD cases HLA-DR positive microglia were also found in the hippocampus, though this was associated with neuritic plaques and tangles suggestive of AD pathology [52]. Involvement of the transenterohinal, cingulate and temporal cortices in PD has also been identified. Activated microglia in these regions also expressed MHC Class II molecules, HLA-DP, DQ and DR [53]. The presence of CD4 (as well as CD8) T lymphocytes within the substantia nigra of PD cases at post-mortem has subsequently been confirmed [54]. In addition, concentrations of interleukin-1β, interleukin-6 and transforming growth factor-α are higher in the striatal regions of post-mortem PD brains compared to controls [55]. Complement proteins are also found with Lewy bodies within this region in PD [56].

In DLB, both complement proteins and microglial interaction are associated with Lewy body containing degenerated neurons on autopsy, suggesting microglial involvement [57]. An increase in activated microglia has also been reported in DLB cases, positively correlating with the number of Lewy bodies also seen regionally [58]. However this was not as high as in those cases with

concomitant senile plaques and a second study has shown a lack of significant microglial activation in the absence of tau neuritic plaques in DLB [59]. The link between microglial activation and pathological protein deposition in both PDD and DLB is therefore not fully established.

7. Evidence from genetic studies

Genetic studies have identified polymorphisms in genes coding IL-1β, TNF-α and Triggering Receptor Expressed on Myeloid cells 2 (TREM2) as risk factors for PD. Up to a doubling of risk has been reported amongst carriers of a genotype of IL-1β that is associated with increased gene expression [60,61]. Those carrying the homozygous variant genotype TNF-α-308, a variant which is thought to be a stronger transcriptional activator, experience doubled risk [60]. Overall the results from these two small studies are consistent with a gene dosing effect for these two powerful cytokines. A rare variant of the microglial receptor TREM2, that leads to loss of function, was found to be another risk factor for PD in a study of 1493 cases compared to 1957 controls [62].

Genome wide association studies (GWAS) provide further evidence for inflammatory pathology in PD. Polymorphisms in HLA regions that code segments of the MHC class II molecule present increased risk. A strong association was found within noncoding intron 1 of HLA-DRA (in a study of 2000 cases and 1986 controls) by Hamza and colleagues [63], with subsequent large-scale meta-analyses of single nucleotide polymorphisms(SNP) confirming associations amid the HLA-DR locus, with both HLA-DRB5 [64] and HLA-DQB1 [65] identified. Wissemann and colleagues [66] found loci that predisposed to, as well as protected from, PD within the same 2000 PD and 1986 control GWAS dataset initially analyzed by Hamza et al. [63], and replicated these in a further 843 cases and 856 controls. The strongest association was again intron 1 of the HLA-DRA region, which regulates gene expression and linked to increased risk. This suggests HLA expression levels may play a key role in determining risk for PD. Indeed subjects homozygous for the G allele in this SNP, were found to have significantly increased MHC class II expression, compared to subjects who did not have a single G allele. In addition, exposure to a common insecticide, pyrethroid, when combined with possession of the GG allele, significantly increased PD risk [67], suggesting a combination of environmental triggers and inflammatory processes may play a part in PD pathology.

Notwithstanding the accumulated genetic evidence in the context of PD, the equivalent associations in DLB have not been established, although methods of investigation may need to be broadened, as studies have been limited so far [68]. Polymorphisms in genes associated with inflammation are also yet to be identified as risk factors for PDD specifically.

8. Evidence from blood biomarkers

Elevated peripheral inflammatory markers both before and after the onset of PD, suggest inflammation is concurrent with the disease. Increased plasma interleukin-6 (IL-6), measured on average 4.3 years before diagnosis, is associated with increased risk of developing PD, with higher levels associated with higher risk [69]. After disease onset, levels of IL-6 [70,71], IL-1β [71] and TNF-α [70] are elevated compared to controls in PD, as is RANTES (regulated on activation, normal T cell expressed and secreted), a chemokine which attracts T-cells. RANTES levels also correlated with motor symptom severity [72]. A change in peripheral blood lymphocyte subsets further suggests a role for the adaptive immune system. A decrease in the overall level of T-helper CD4 cells but a rise in the subset of activated T-helper cells is reported in PD cases compared to controls [73].

In PDD, high sensitivity CRP is increased compared to controls, but a significant elevation was not found in PDD compared to PD [74]. Peripheral markers suggestive of inflammation are yet to be found in DLB. Therefore the blood biomarkers evidence for inflammation in LBDs is inconclusive.

9. Evidence from cerebrospinal fluid biomarkers

Attempts to identify a reliable cerebrospinal fluid (CSF) biomarker for PD or PDD have so far been inconsistent. The main candidates include total α-synuclein, Aβ42, and β-Glucocerebrosidase [75]. Inflammatory cytokines TNF-α [76], IL-6 [77,78] and IL-1β [71,77] have also been investigated with raised levels seen in the CSF of PD cases compared to controls. IL-1β levels in the CSF were associated with raised α-synuclein oligomers also in the CSF, suggesting a direct link with protein deposition [71].

In a study of 22 cases of PD, IL-6 was found to associate inversely with disease severity as assessed by the UPDRS [78]. In a larger study of 62 cases, IL-6 was elevated in cases of PD with cognitive impairment compared to those without, the levels being negatively correlated to cognitive function. TNF-α and Interferon γ levels were however reduced in those with cognitive impairment in PD compared to control subjects [79]. A rise in the fractalkine:Aβ42 ratio in CSF is also associated with motor severity of PD (again measured by UPDRS) but not with disease duration [80]. An increase in this ratio could suggest increased inflammatory signaling and microglial activation. An increase in Leucine rich α2-glycoprotein (LRG), thought to be a marker of inflammation, is reported in the CSF and post-mortem tissue of PDD and DLB cases, compared to controls [81].

The focus in DLB has been on the variations of Aβ peptides and tau as well as α-synuclein; a combination of biomarkers may be the best route to increase specificity and sensitivity [82,83]. The inflammatory marker Procalcitonin has been found to be significantly raised in dementia subjects within the CSF, compared to controls, with the highest median level found in DLB cases [84].

10. Evidence from epidemiological studies

There is limited support for neuroinflammation in PD from epidemiology studies. A meta-analysis of the association of NSAIDs and the risk of developing PD, showed a 15% reduction in incidence among users of non-aspirin NSAIDS, with analysis of ibuprofen alone showing a stronger protective effect. This effect was more pronounced among regular users [85]. Whether PDD incidence was lower in those who developed PD despite taking NSAIDS was not considered.

A further meta-analysis showed conflicting results with no overall protective effect, however there were methodological differences including the inclusion of aspirin and studies where NSAID exposure was entirely within a 1 year of the diagnosis of PD. Nevertheless a slight protective effect for ibuprofen in lowering the risk of PD was still confirmed [86]. The evidence from these studies is however difficult to interpret because of variations in the drugs investigated, the duration of the drug treatment and the timing of administration in relation to disease onset.

Whether NSAIDs could reduce the risk of developing DLB or protect those with PD from developing dementia, has not yet been established.

11. A role for the adaptive immune system

Despite the evidence of microglial activation and an interaction between α-synuclein and microglia, the precise mechanism and

whether it is always detrimental to neurons remains unclear. A paucity of the relationship between Lewy bodies and antigen presenting activated microglia in post mortem studies was reported by Imamura et al. [53], indeed there was only a 20% association. This would suggest that Lewy bodies alone are not sufficient in themselves to trigger antigen presentation by microglia. In addition, increasing neuronal loss in the substantia nigra with lengthening disease duration was not associated with an increase in microglial activation, implying a steady rather than escalating inflammatory response [87].

Orr and colleagues [87] also demonstrated that substantia nigra neurons were immunopositive for IgG in PD, whereas control cases' substantia nigra neurons as well as the visual cortex of PD cases showed negative immunoreactivity. Neuronal IgG labelling related to the degree of neuronal loss and microglial activation, with the authors suggesting humoral immune system involvement in the selective destruction of substantia nigra neurons.

Given that the MHC class II complex has also been shown to be key in dopamine neuronal cell loss in mouse models [50], it may be that an adaptive immune response is the final path to neuronal loss, following a switch in microglia function from protective to deleterious. Consistent with this theory is the genetic risk associated with HLA class II gene variation previously described, as well as the alteration in peripheral lymphocyte subsets found in PD cases [73], and the evidence that B and T lymphocyte infiltration of the substantia nigra is found at post mortem [54] and in a mouse model of α-synuclein overexpression [35].

It is possible initial protein clearance by microglia could be switched to a more harmful toxic function involving recruitment of the adaptive response ultimately leading to neuronal degeneration. For example due to peripheral inflammation or increased vulnerability of microglia through ageing. The timing of treatment initiation would be key in such circumstances.

12. Conclusion and future directions

Evidence for the role of neuroinflammation in LBDs continues to accumulate, building on the evidence of neuroinflammation in AD and PD. Imaging studies lead the way in supporting neuroinflammation as a key part of the pathogen process in LBDs, supported by pathological and biomarker evidence, though mostly in PDD. Future studies are required to further establish the presence of inflammation in DLB including imaging, genetic and biomarker studies.

Involvement of microglia in LBDs is signified by the presence of activation years before neuronal death as revealed by *in vivo* imaging, as well as after cell loss in pathology specimens. Microglial involvement is also supported by evidence of the activation of microglia by α-synuclein. Levels of activation however appear to remain relatively stable, which could indicate initiation and propagation of the disease process by microglia or alternatively a protective function that is eventually overcome. In order to understand how inflammation affects disease progression in Lewy body dementia, studies need to try and link the nature and extent of microglial activation with important indicators of disease severity such as structural brain changes, protein deposition and the onset and progression of key cognitive and non-cognitive symptoms through longitudinal studies in established disease and in those at risk.

Documentation of author roles

Ajenthan Surendranathan wrote the manuscript which was reviewed and critiqued by James Rowe and John O'Brien.

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Appendix 3

Diagnostic and Management Pathway Study

Patient Information Sheets and Consent Forms

What will happen next?
If you definitely do not want to take part, please complete and return the reply slip in the envelope provided – no stamp is needed. If you prefer, you can telephone a member of the DeNDRoN Eastern team on: 01223 218620 to let us know that you do not want to take part.

If we do not hear from you, we will telephone you to check whether or not you are interested in helping us with this study. If so, we will arrange to visit you, either at home or in the clinic, whichever you prefer. During this visit you will have the opportunity to ask any questions about the study before you make a decision. If you decide to take part, we will discuss a consent form with you and ask you to sign it. Once you have given your consent, we will not need to contact you again.

Do I have to take part?
It is up to you to decide whether or not to take part. You do not have to give a reason if you do not want to be involved. Whatever you decide will have no effect on the care you receive now or in the future. You are free to change your mind at any time and do not need to give us a reason.

How can you contact us?
If you would like further information about the study, please contact the research team - Allison Bentley or Dr Ajenthan Surendranathan on 01223 767037.

Cambridge University Hospitals NHS
NHS Foundation Trust

Diagnosing and managing memory problems: a review of current practice

Patient information sheet

You are being invited to take part in a research study. This leaflet explains why the research is being done and what taking part will involve for you. Please read the following information carefully and discuss it with others if you wish. You can then talk to the researchers before you decide whether to go ahead. Thank you for reading this.

UNIVERSITY OF CAMBRIDGE

What is the study about?

Memory problems are common in older people and can have a number of different causes but can be difficult to make an exact diagnosis. This study aims to improve the prompt diagnosis of memory problems. In order to improve the service, we need to start by looking at current practice. We want to include people who have different types of memory problems. You have been sent this leaflet because you have seen a specialist about your memory problems.

What does taking part involve?

This study would only involve a review of medical notes. Before we can do this, we need your permission; you will not be asked to do anything else. It is important that as many people as possible give their consent so that we get an accurate picture of current practice. If you are willing to help with the study, we will need to visit you once to answer any questions you have about the study and to ask you to sign a consent form. Following this visit we will not need to contact you again.

What difference will it make?

The study will not make any difference to your care but we hope it will help improve services for other people with memory problems.

Personal information policy

We understand that you may be concerned about privacy. We would like to reassure you that all information from your medical records will be treated in the strictest confidence. The information collected will be stored securely in locked cabinets or on password protected computer systems. Each person who takes part will be given an ID number and this will be used when recording information from your medical records. Your personal details, for example your name and date of birth, will be kept in a separate file. All members of the research team will have appropriate training and will have signed a confidentiality agreement. The study has been approved by the National Research Ethics Service (NRES Committee North East - Newcastle & North Tyneside 1).

Who is organising and paying for the study?

This is a National Institute for Health Research (NIHR) Programme Grant for Applied Research project. The research team is based at the Universities of Newcastle and Cambridge.

If you have any concerns or complaints about the research, we will do our best to resolve them. Please contact:

Patient Advice and Liaison Service (PALS):
Tel: 01223 726774 (office hours) or free-phone 0800 052 1411. A confidential e-mail service is also available at: pals@cpft.nhs.uk

UNIVERSITY OF CAMBRIDGE

Cambridge University Hospitals NHS
NHS Foundation Trust

CONSENT FORM FOR PATIENTS

Diagnosing and managing memory problems: a review of current practice

I ..... **(name)**

of ……..

……**(address)**

Please initial

I have read the information sheet giving details of this study, have been given a copy to keep and have had the opportunity to ask questions. ☐

I understand that my participation is voluntary and I can withdraw consent at any time without giving any reason and without my medical care or legal rights being affected. ☐

I understand that this consent form and data collected during the study may be looked at by responsible individuals from the research Sponsor (Northumberland, Tyne & Wear NHS Foundation Trust) or its representatives or from regulatory or ethical authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

I understand that sections of my medical notes, including my GP notes, may be looked at and information taken from them used in this research. I give permission for the researchers to have such access to my records. ☐

I give permission for information concerning me to be held by Newcastle University. I understand that records will be confidential and will be stored securely on systems within the NHS and University. ☐

I consent to take part in this study. ☐

Signed..... …… **Date**.....

Consented by ..... …….(signed) **Date**.....

Print Name .....

What will happen next?
If you definitely do not want to take part, please complete and return the reply slip in the envelope provided – no stamp is needed. If you prefer, you can telephone a member of the DeNDRoN team East Anglia on: 01223 218620 to let us know that you do not want to take part.

If we do not hear from you, we will telephone you to check whether or not you are interested in helping us with this study. If so, we will arrange to visit you, either at home or in the clinic, whichever you prefer. During this visit you will have the opportunity to ask any questions about the study before you make a decision. If you decide to take part, we will discuss a consent form with you and ask you to sign it. Once you have given your consent, we will not need to contact you again.

Do I have to take part?
It is up to you to decide whether or not to take part. You do not have to give a reason if you do not want to be involved. Whatever you decide will have no effect on the care you receive now or in the future. You are free to change your mind at any time and do not need to give us a reason.

How can you contact us?
If you would like further information, please contact Anne Maule on 0191 208 1310.

Cambridge University Hospitals NHS
NHS Foundation Trust

Diagnosing and managing memory problems: a review of current practice

Patient information sheet

You are being invited to take part in a research study. This leaflet explains why the research is being done and what taking part will involve for you. Please read the following information carefully and discuss it with others if you wish. You can then talk to the researchers before you decide whether to go ahead. Thank you for reading this.

UNIVERSITY OF CAMBRIDGE

What is the study about?

Memory problems are common in older people and can have a number of different causes but can be difficult to make an exact diagnosis. This study aims to improve the prompt diagnosis of memory problems. In order to improve the service, we need to start by looking at current practice. We want to include people who have different types of memory problems. You have been sent this leaflet because you have seen a specialist about your memory problems.

What does taking part involve?

This study would only involve a review of medical notes. Before we can do this, we need your permission; you will not be asked to do anything else. It is important that as many people as possible give their consent so that we get an accurate picture of current practice. If you are willing to help with the study, we will need to visit you once to answer any questions you have about the study and to ask you to sign a consent form. Following this visit we will not need to contact you again.

What difference will it make?

The study will not make any difference to your care but we hope it will help improve services for other people with memory problems.

Personal information policy

We understand that you may be concerned about privacy. We would like to reassure you that all information from your medical records will be treated in the strictest confidence. The information collected will be stored securely in locked cabinets or on password protected computer systems. Each person who takes part will be given an ID number and this will be used when recording information from your medical records. Your personal details, for example your name and date of birth, will be kept in a separate file. All members of the research team will have appropriate training and will have signed a confidentiality agreement. The study has been approved by the National Research Ethics Service (NRES Committee North East - Newcastle & North Tyneside 1).

Who is organising and paying for the study?

This is a National Institute for Health Research (NIHR) Programme Grant for Applied Research project. The research team is based at the Universities of Newcastle and Cambridge.

If you have any concerns or complaints about the research, we will do our best to resolve them. Please contact:

Philip Kyle, Programme Manager
Telephone: 0191 208 1322
Email: Philip.Kyle@ncl.ac.uk

Institute for Ageing and Health, Newcastle University
Campus for Ageing and Vitality
Newcastle upon Tyne, NE4 5PL

UNIVERSITY OF CAMBRIDGE

Cambridge University Hospitals NHS
NHS Foundation Trust

CONSENT FORM FOR PATIENTS

Diagnosing and managing memory problems in Parkinson's disease: a review of current practice

I ..... **(name)**

of ……..

……**(address)**

Please initial

I have read the information sheet giving details of this study, have been given a copy to keep and have had the opportunity to ask questions. ☐

I understand that my participation is voluntary and I can withdraw consent at any time without giving any reason and without my medical care or legal rights being affected. ☐

I understand that this consent form and data collected during the study may be looked at by responsible individuals from the research Sponsor (Northumberland, Tyne & Wear NHS Foundation Trust) or its representatives or from regulatory or ethical authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

I understand that sections of my medical notes, including my GP notes, may be looked at and information taken from them used in this research. I give permission for the researchers to have such access to my records. ☐

I give permission for information concerning me to be held by Newcastle University. I understand that records will be confidential and will be stored securely on systems within the NHS and University. ☐

I consent to take part in this study. ☐

Signed..... …… **Date**.....

Consented by ..... …….(signed) **Date**.....

Print Name ..... ……..

Appendix 4

Neuroinflammation Study

Patient Information Sheets and Consent Forms

Cambridge University Hospitals NHS
NHS Foundation Trust

UNIVERSITY OF CAMBRIDGE

Information Sheet for Patients and their Informants

Neuroimaging of Inflammation in MemoRy and Other Disorders (NIMROD)

You are being invited to take part in a research study. This leaflet explains why the research is being done and what taking part will involve. Please read the following information carefully and discuss it with others if you wish. You can talk to the researchers before you decide.

If you decide to take part, after reading this information leaflet, please sign the consent form.

If you decide not to take part it will not affect the standard of health care you receive in any way.

What is the purpose of the study?

There are several different causes of memory problems in later life, including a condition called Mild Cognitive Impairment as well as different types of dementia like Alzheimer's disease, Frontotemporal dementia, Lewy body dementia, Progressive supranuclear palsy and Vascular dementia. Older people with depression and with Parkinson's disease can also have memory problems.

While considerable progress has been made over the last decade in understanding some of the brain protein and other changes associated with memory problems and dementia, a lot is still not known. For example, why some people with memory problems get worse at a faster rate than others is not clear. It has been established that mild inflammatory changes (brain inflammation) are associated with some of these disorders, but the questions whether it is present in all of them, and if so precisely where and how it changes with time have received little research attention. This is important as we do not know how much inflammation is the result of disease and how much it may be involved as a cause. If it is a cause, then this is extremely important because it may be possible to develop new treatments to help prevent memory problems getting worse. It may also be possible to use measures of inflammation to predict groups of individuals who may be more at risk of declining more quickly than others.

Brain imaging is an important method to investigate brain structure and function. Magnetic Resonance Imaging (MRI) can be used to look at brain structure and function in great detail. In addition, Positron Emission Tomography (PET) imaging can be used to help visualise some kinds of damaged protein often found in people with memory and other problems (amyloid using PIB PET imaging and tau using AV-1451 PET imaging), and also can help detect the presence of inflammation in the brain (PK11195 PET). Further, the presence of illnesses, including inflammation, leaves tell-tale changes in the blood and cerebro-spinal fluid, the clear fluid that bathes the brain and spinal cord. In addition there are known to be genetic markers found in the blood that are associated with increased or decreased risk of dementia.

This study looks for the presence of damaged protein, genetic markers, inflammation and changes in brain structure and function in people with a range of disorders that affect their thinking, attention and memory as well as suitable control subjects without such impairments. We will compare them to see how they differ on the tests and scans, to understand the causes and effects of dementia and related illnesses.

Why have I been invited?

You have been selected because you have either been diagnosed with a neurodegenerative disease or with depression or because you have symptoms that are suggestive of such a disorder.

Do I have to take part?

It is up to you to decide whether to join the study. If you decide you do not wish to take part it would not affect the standard of health care you receive in any way.

If you agree to take part we will ask you to sign a consent form.

You are free to withdraw at any time without giving a reason. This would not affect the standard of health care you receive in any way.

What will I be asked to do?

The study includes the following types of test, although not everyone will necessarily be asked to do all parts of the study:

1. A clinical assessment, including memory and other cognitive tests.
2. A blood test.
3. An MRI brain scan
4. Either one, two or three PET scans
5. Some people may also be invited for a lumbar puncture, to examine spinal fluid.

Participants will have tests of memory, language, vision and attention, which take about one and a half hours to complete. We will ask someone who knows you well to have a short interview to answer questions and complete a couple of questionnaires about how you are and how you are coping with everyday life. This would be repeated every year for the duration of the study (up to 3 years) and can be carried out either at your home or at Addenbrooke's Hospital, whichever you prefer. We may use an audio device to record some of your answers. This will only be used as a supplement to written notes to ensure accuracy.

We would carry out a brief physical examination, which could either be as part of a normal clinical attendance or combined with one of the other research visits. This would be repeated annually. We would also take a blood sample (about 80 ml, or 2 eggcups full). These take about 10 minutes.

We propose to undertake up to three PET scans at Addenbrooke's Hospital in the Wolfson Brain Imaging Centre (WBIC) or in the Hospital's PET/CT Department. On each occasion you will be in the Department for approximately 2 hours, with the scan itself taking 45 minutes in the case of PIB PET and 90 minutes for the others (PK11195 and AV-1451 PET). For each PET scan you will have an injection of about a teaspoon of short lasting radioactive liquid. The radiation dose for each PIB and PK11195 PET scan (2.7 – 3.0 milliSieverts) is similar to the radiation dose we each experience from radiation in the environment during one year living in the East Anglia region, while the radiation dose from AV-1451 PET is 9.3 milliSieverts, which is similar to 3½ years' environmental exposure here. The injected radioactivity fades away naturally over a few hours and you can leave the Scanning Centre or Department as soon as the scan is finished.

In all cases our staff would communicate with you throughout the scan to check that you stay comfortable. You could end the scan at any point.

The MRI brain scan will take around an hour and also takes place at the WBIC. This is to look at the size, shape and 'wiring' of the brain. It may be possible to arrange for this to take place on the same day as one of the PET scans. Though MRI scanning is generally very safe, there are certain circumstances where it must be avoided. We will go though a checklist

to ask about metal objects attached to or inside your body (e.g. stents, shrapnel, plated fractures,) or electronic devices (e.g. heart pace-maker). Many such items (most modern cardiac stents, for instance) are designed to be MRI safe. Being scanned requires you to lie still and relaxed on a bed in the scanner's 'tunnel'. This 'tunnel' is quite narrow so please let us know if you have experienced claustrophobia in small spaces. It can be noisy but earplugs are supplied and you can also have your own choice of music played over headphones if you wish. As with the PET scans, the technician performing the MRI scan would communicate with you throughout the scan to check that you stay comfortable. It can be stopped at any point, but takes up to one hour to complete.

Some participants would also be invited for a lumbar puncture, on another visit, to take a small volume (about 15ml, three teaspoonsful) of the spinal fluid that has bathed the brain before travelling down the spine. It can tell us a lot about what is happening in the brain. A separate information sheet is available on lumbar puncture, as it is not relevant to everyone, and is an optional part of the study.

What are the possible benefits of taking part?

This is not a trial of any drug or other treatment and there is no direct benefit to you from taking part in this study. However if you do take part you will be making a significant contribution to medical knowledge and the challenge of dementia especially.

Expenses

If you take part in this study, we would cover all necessary travel expenses and if it would help we would arrange transport by taxi for you to come to the hospital and go home.

Will my taking part in the study be kept confidential?

If you do take part in the study, all information provided to us and the results of studies would be treated confidentially. It will be stored securely in locked cabinets or on password protected computer systems, under the supervision of the Chief investigators. We will retain the data for over 10 years. We will ask for your permission to share your data and scans in an anonymised way with collaborators, now and in the future, including researchers in the NHS, Medical Research Council, University and National Institute for Health Research. The NHS is trying to improve the quality of clinical and research standards. This is being achieved through 'clinical governance'. As part of this process, this study may be reviewed by a clinical governance team. Such a team would need to look at our records to make sure that the research was carried out in accordance with proper procedures.

What if there is a problem?

Although the PET scans are for research purposes only, the MRI scan will be routinely reported by a specialist radiologist. Occasionally, brain scanning and other tests reveal a medical problem that was not expected. If this happens, we will inform you, and (if you agree) we would write to your General Practitioner (GP) and arrange for any necessary NHS follow up.

We have also arranged insurance, in the unlikely event of any problems, without affecting your statutory rights. If you have any concerns or comments related to your participation in this study, you could contact the Chief Investigator (details below) or the Patient Advisory and Liaison Service (PALS) at Box 53, Cambridge University Hospitals, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0QQ, telephone 01223 216 756, e-mail pals@addenbrookes.nhs.uk.

Who is organising and funding the research?

The study is primarily funded by the NIHR (National Institute for Health Research) Biomedical Research Unit. The research team are based at the Departments of Psychiatry and Clinical Neurosciences at the University of Cambridge and Cambridge University Hospitals NHS Foundation Trust (Addenbrooke's Hospital).

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect the participants' interests. This study has been reviewed and given a favourable opinion by the East of England – Cambridge Central Research Ethics Committee.

Further information

If you would like further information please contact Professor John O'Brien, or any member of the research team (details below).

What will happen next?

The next step will be a telephone call from one of the researchers. If you are interested in helping with the study, they will arrange to visit you at home. This will give you a chance to ask any questions about the study and your taking part before you make a decision. If you do decide to take part, the researcher will discuss a consent form with you and ask you to sign it. It is up to you to decide whether to take part or not. You do not have to give a reason if you decide not to be involved. If you change your mind you can withdraw from the study at any time without giving a reason. You will be given a copy of this leaflet to keep.

The research team should contact you in the next week or so. If, at any time, you need to get in touch with someone, you can contact us:

Professor John T O'Brien (Chief Investigator)
Department of Psychiatry,
University of Cambridge,
Level E4, Box 189,
Addenbrooke's Hospital,
Hills Road,
Cambridge, CB2 0SP
Tel: +44 (0)1223 760682

Dr James B Rowe (co-Chief Investigator)
Department of Clinical Neurosciences,
University of Cambridge,
Herchel Smith Building,
Forvie Site, Robinson Way,
Addenbrooke's Hospital ,
Cambridge, CB2 0SZ
Telephone: +44 (0) 1223 273 630
Secretary: +44 (0) 1223 760 696

Robert Arnold (Research Assistant)
Herchel Smith Building,
Forvie Site, Robinson Way,
Cambridge, CB2 0SZ
Telephone: 01223 768003
Email: rja39@medschl.cam.ac.uk

Cambridge University Hospitals NHS
NHS Foundation Trust

UNIVERSITY OF CAMBRIDGE

Consent Form for Patients

Neuroimaging of Inflammation in MemoRy and Other Disorders (NIMROD)

I, ...**(name)**

of .....

.....**(address)**

consent to taking part in the NIMROD study.

Please tick

I have read the information sheet giving details of this study, have been given a copy to keep and have had the opportunity to ask questions.	☐
I understand that my participation is voluntary and I can withdraw consent at any time without giving any reason and without my medical care or legal rights being affected.	☐
I understand that sections of any of my medical notes may be looked at and information taken from them used in this research, or in the monitoring of the research by clinical governance staff. I give permission for the researchers and clinical governance staff to have such access to my records.	☐
I understand that my tissue samples will be tested for genetic factors and I agree to this.	☐
I give permission for information concerning me to be held by the University of Cambridge. I understand that records will be confidential and will be stored securely on systems within the NHS and University.	☐
I understand that my GP will be informed of my participation in this study, and give permission for this.	☐
I give my permission that in the unlikely event that an abnormality is discovered my GP and I will be informed.	☐
In the possible event of my losing mental capacity to give informed consent during this study, I wish it to be noted that I am minded to continue in the study.	☐
I consent for my data and tissue samples to be used in similar studies.	☐

Signed ..... **Date**.....

Consented by ..... (sign) **Date**.....

Print name .....

When completed, original to be kept in research file, 1 copy for Participant

Appendix 5

Author Contribution

Within this appendix are the specific contributions made by myself for each chapter.

Chapter One: Lewy Body Dementia Diagnosis - Background

I carried out all the work in this chapter, including:

- creation of the concepts for the literature search, and
- the literature search itself.

Chapter Two: Lewy Body Dementia Diagnosis – Methods

Chapters two to four were carried out as part of the Diamond Lewy Project, a large study based in both East Anglia and the North East which was funded by the NIHR Grant Reference Number DTC-RP-PG-0311-12001). These chapters were part of "Diamond Lewy Work Package 1" in East Anglia.

In this chapter, I

- created the hypotheses for testing,
- approached and obtained consent from the clinicians for access to their clinic lists for the prevalence survey data collection,
- carried out a third (approximately) of the data collection for the prevalence survey (screening of medical records),
- supervised the remaining data collection for the prevalence survey and checked the data for quality,
- amended the case report form sections to ensure they contained the appropriate data to test the hypotheses,
- assisted with the recruitment of patients for the diagnostic survey (the detailed medical notes' analysis),
- carried out about a third (approximately) of the data collection for the diagnostic survey,
- supervised the remainder of the data collection for the diagnostic survey, and
- checked the entire database containing the data from the case report forms (CRFs) against each CRF to ensure data transcription was accurate and I made corrections as necessary.

Chapter 3: Lewy Body Dementia Diagnosis – Results

In this chapter, I

- chose the data to be analysed from the data collection for both the prevalence survey and diagnostic survey statistical analyses,
- chose the appropriate statistical test for the analysis of each dataset, except the choice of the Wilson's test for the prevalence confidence interval which was made after discussion with the Diamond Lewy Study statistician, and
- carried out all the data analysis.

Chapter 4: Lewy Body Dementia Diagnosis – Discussion

I carried out all the work in this chapter. The published paper ("Clinical Prevalence of Lewy Body Dementia", attached as Appendix 1) where I am joint lead author, was an amalgamation of the prevalence survey results in East Anglia (which are in my thesis) and the prevalence survey results in the North East (which are not in my thesis).

Chapter 5: Inflammation in Lewy Body Dementia – Background Literature Review

I carried out all the work in this chapter, including:

- creation of the concepts for the literature search, and
- the literature search itself.

The published paper ("Neuroinflammation in Lewy Body Dementia", attached as Appendix 2), where I am lead author and carried out the literature search and wrote the manuscript, has been updated in this chapter by a further literature search carried out in March 2018 as detailed in the chapter.

Chapter 6: Inflammation in Dementia with Lewy Bodies – Introduction and Methods

Chapters 6-8 were carried out as part of the NIMROD study (primarily funded by the NIHR National Institute for Health Research Cambridge Biomedical Research Centre (grant

number RG64473), which is a large multimodal imaging study of different dementia subtypes. I was responsible for the dementia with Lewy bodies group within that study.

In this chapter, I

- created the hypotheses for testing,
- obtained the participants' blood samples and processed them for future analysis,
- identified the inflammatory markers to be tested from the literature search,
- collated the relevant blood samples and organised their processing,
- learned support vector machine methodology, and how to code for it on R

and for the majority of the DLB participants, I

- identified and carried out their recruitment,
- obtained their clinical history,
- arranged and provided clinical supervision for their MRI and PET scans, and
- carried out their clinical examinations, including UPDRS.

Chapter 7: Inflammation in Dementia with Lewy Bodies – Results

In this chapter, I

- chose the appropriate data to analyse in order to test the hypotheses,
- identified the most appropriate statistical analysis,
- carried out the statistical analysis, and
- carried out the support vector machine analysis.

The PET modelling was carried out by Young Hong and Tim Fryer. They provided regional BP_{ND} values from each participant for each PK11195 and Pittsburgh Compound B scan so that I could carry out the statistical analysis. The cytokine analysis was carried out by Core Biochemical Assay Laboratory, Cambridge University Hospital, who provided the assay results for each participant so that I could carry out the statistical analysis.

Chapter 8: Inflammation in Dementia with Lewy Bodies – Discussion

I carried out all the work in this chapter.

Chapter 9: Conclusion and Future Work

I carried out all the work in this chapter.