Biomarker and pathology studies in neurodegenerative cognitive impairment

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But learn to learn, and try to learn for what.

B. Brecht

Scientific environment

This project started while I was a medical student at the Research Program at the University of Bergen. I have been part of the Dementia Study of Western Norway (Demvest) study group from the start in 2005. The majority of the work has been conducted at Haraldsplass Deaconess Hospital, in combination with a 50% resident position with the department of Geriatric Medicine. In addition to Haraldsplass Deaconess Hospital my affiliations include Department of Clinical Medicine at the University of Bergen, The Kavli Centre for Geriatrics and Dementia and SESAM – Centre for Age-related Medicine.

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Abstract

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Background: Dementia is a major cause of functional impairment and early death in older age groups. Neurodegenerative disorders are the most common cause of dementia. The most frequent neuropathological lesions include neurofibrillary tangles and senile plaques, hallmark lesions for Alzheimer´s disease (AD), and Lewy body pathology, which characterize Lewy body disease (LBD). Clinically, the neuropathological entity LBD can present as either Parkinson´s disease (PD) or dementia with Lewy bodies (DLB), differentiated on the basis of the presenting symptoms being either motor or cognitive. While the majority of LBD patients develop both motor symptoms and cognitive impairment, some patients with clinical PD will never experience cognitive impairment and likewise some patients with DLB will never develop motor symptoms.

Similarly the clinical presentation of AD is also heterogeneous, for instance, the highly variable occurrence of neuropsychiatric symptoms and rate of progression. These differences have a major impact on quality of life for patients and carers, as well as health care costs, but their mechanisms and neuropathological underpinnings are poorly understood. Furthermore the correlation between clinical diagnosis and neuropathological findings is relatively low, and LBD patients presenting with cognitive impairment particularly risk being misclassified as AD. This highlight the need for more precise biomarkers for these clinical syndromes that can be implemented at the start of and during the course of the disease.

Biomarkers may inform about disease pathology, thus paving the way for new treatment, they increase diagnostic accuracy and aid in setting a prognosis. Biomarkers are needed in the selection of patients for treatment studies and to identify which patients should benefit from new treatment when available. The cerebrospinal fluid (CSF) biomarkers beta-amyloid 42 (abeta42), total tau (t-tau) and tau protein phosphorylated at amino acid 181 (p-tau181) reflect key AD pathologies. The Lewy

bodies found in LBD are composed mainly of the protein α-synuclein. α-synuclein is reduced in CSF in LBD, but with considerable overlap between LBD, controls and other disease groups.

Aim: The main aim of this thesis was to increase understanding of pathological mechanisms underlying important clinical features in neurodegenerative cognitive impairment, by exploring the associations between clinical presentation and biomarkers and pathology. The first objective was to explore the association between AD pathology CSF markers and neuropsychiatric symptoms in newly diagnosed AD patients; secondly to assess the association between CSF markers of AD and LBD pathology and early cognitive impairment in PD; thirdly to examine the correlation between clinical diagnosis of DLB and Lewy body pathology at autopsy.

Methods: This is a clinical translational neuroscience project based on two clinical cohort studies. The dementia Study of Western Norway (Demvest) included newly diagnosed dementia patients from specialist clinics in geriatric medicine and old age psychiatry in Western Norway. The Parkinson´s Progression Markers Initiative (PPMI) is an international multicentre study, including newly diagnosed PD patients and healthy controls. A comprehensive battery of neuropsychological tests, a structured neuropsychiatric evaluation, clinical examination, and imaging were part of both studies. CSF sampling was done according to standardized protocols and CSF was analysed using commercially available immunoassays. In the Demvest study, participants were recruited for brain donation, and autopsy results were obtained applying commonly used neuropathological protocols and diagnostic criteria.

Results: We undertook three specific studies to investigate objective I, II and III. In study I, apathy in patients with early Alzheimer´s disease correlated with t-tau and p-tau181 concentrations in CSF, higher values being associated with more severe apathy. There were no associations between depression or psychosis and agitation and CSF markers.

In study II, decreased CSF α-synuclein in newly diagnosed PD-patients without dementia correlated with impaired global cognition and impairment of executive functions and attention. CSF abeta42 was decreased in PD with mild cognitive impairment compared with controls after adjusting for covariates. No correlations were found between memory or visuospatial functions and CSF markers.

Study III examined autopsy results of 56 patients followed from dementia diagnosis to death. 20 patients received a pathological diagnosis of LBD; the corresponding clinical diagnosis were probable DLB (n=11), Parkinson´s disease with dementia (PDD) (n=5) and probable or possible AD (n=4). Of the 56, 14 patients received a clinical diagnosis of probable DLB, 11 of these had pathological LBD and three AD. Sensitivity, specificity, positive and negative predictive values of a clinical DLB diagnosis were 73%, 93%, 70%, and 90% respectively.

Conclusions and implications: We have reported a novel association between neuropsychiatric symptoms and CSF biomarkers reflecting core AD pathology. The relationship between t-tau and p-tau181 and apathy may reflect an association between neurofibrillary tangle pathology and apathy in early AD.

Cognitive impairment in early PD was associated with biomarkers of both Lewy body and AD pathology. 18 of 20 LBD patients in the Demvest study had Braak neurofibrillary tangle stage IV or higher, representing severe AD pathology at autopsy. Thus our findings suggest a role for AD pathology in both early and established LBD.

Accurate diagnosis is crucial for clinical practice and research. With a sensitivity of 73%, the clinical 2005 DLB criteria are not sensitive enough. More than one in four DLB patients were not identified even when structured rating scales for core DLB symptoms were applied. We regard a specificity of 93% as satisfactory. Our results illustrate that not all DLB patients fulfil the 2005 DLB criteria at disease presentation, highlighting the need for re-evaluation of the diagnosis if new symptoms appear.

Studies applying the most recent 2017 DLB criteria will show if this revision has increased sensitivity without decreasing specificity.

List of publications

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Table of contents

Abbreviations

Abeta peptides: Beta-amyloid peptides, abeta42 has 42 amino acids, abeta40 has 40 amino acids.
AD-MCI: MCI patients who develop AD
AD: Alzheimer's disease
ADL: Activities of daily living
APOE: The gene that codes for Apolipoprotein E
CERAD: The Consortium to Establish a Registry for Alzheimer´s disease
CSF: Cerebrospinal fluid
CV: Coefficient of variation
DAT imaging: Dopamine Transporter Imaging
Demvest: The Dementia Study of Western Norway
DLB-MCI: MCI patients who develop DLB
DLB: Dementia with Lewy bodies
DSM: Diagnostic and Statistical Manual of Mental Disorders
ELISA: Enzyme linked immunosorbent assay
FDG-PET: ^{18}F-Fluorodeoxyglucose positron emission tomography
FTD: Frontotemporal dementia
GBA: The gene that codes for the enzyme beta-glucocerebrosidase
HLVT: Hopkins Verbal Learning Test
ICD-10: International Statistical Classification of Diseases and Related Health Problems, 10th edition
IQCODE: Informant Questionnaire on Cognitive Decline in the Elderly
LBD: Lewy body disease – Parkinson's disease and dementia with Lewy bodies
LRRK2: Leucine-rich repeat kinase 2
MCI: Mild cognitive impairment
mL: Milliliter
MMSE: Mini-Mental State Evaluation
MoCA: Montreal Cognitive Assessment
MSA: Multiple system atrophy

NPI: Neuropsychiatric Inventory

OR: Odds ratio

p-tau181: Tau protein phosphorylated at amino acid number 181

PD-MCI: Parkinson's disease with mild cognitive impairment

PD: Parkinson's disease

PDD: Parkinson's disease with dementia

PiB-PET: ^{11}C-PiB, Pittsburgh Compound B. PET tracer that bind to beta-amyloid plaques

PPMI: Parkinson`s Progression Markers Initiative

PSA: Progressive supranuclear palsy

RBD: REM Sleep Behavior Disorder

SD: Standard deviation

t-tau: Total tau protein

UPDRS: Unified Parkinson´s Disease Rating Scale

VaD: Vascular dementia

1. Introduction

1.1 Dementia

Dementia is common. The number of people living with dementia was estimated to be 47 million in 2015 and is expected to increase to 66 million by 2030 and 131 million by 2050[1]. Whilst the prevalence is increasing due to aging populations, there is some evidence that the incidence is decreasing in industrialized countries[1-3]. This might be caused by both decreased incidence and better treatment of dementia risk factors such as hypertension, diabetes, smoking, and cerebrovascular disease and increased exposure to protective influences such as a healthy lifestyle, balanced diet and education[1, 3]. Dementia has major consequences for both the individuals affected, and their family and carers, as well as being associated with high costs in both primary and secondary health care settings[1].

According to the clinical International Statistical Classifications of Diseases and Health Related Problems (ICD-10) by the World Health Organization[4];

> *Dementia is a syndrome due to disease of the brain, usually of a chronic or progressive nature, in which there is disturbance of multiple higher cortical functions, including memory, thinking, orientation, comprehension, calculation, learning capacity, language, and judgement. Consciousness is not clouded. The impairments of cognitive function are commonly accompanied, and occasionally preceded, by deterioration in emotional control, social behaviour, or motivation*.

It is also specified that the syndrome affects activities of daily living. AD, vascular dementia (VaD), DLB and mixed dementia are the most common causes of dementia[1]. Frontotemporal dementia (FTD) is less common overall, but relatively more common in young people. Neuropathologically DLB and PD are both characterized by Lewy

body pathology; therefore they are commonly referred to as Lewy body diseases (LBD)[5].

AD and LBD are neurodegenerative disorders with heterogeneous clinical presentations. There are major differences in symptoms and disease course between individuals with the same condition. The mechanisms and neuropathology underlying these differences are poorly understood. Interestingly, AD and LBD with dementia share many clinical features, furthermore, at the neuropathological examination the combination of AD and LBD pathology is common.

1.2 Biomarkers

A biomarker can be defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention"[6]. Biomarkers can be used to predict future disease at the pre-clinical stage, give information about prognosis, increase diagnostic accuracy or predict treatment response[7]. Biomarkers and detailed neuropathological examinations can aid in elucidating the differences and similarities between AD and LBD, and support the clinical management and development of novel treatment. Development of effective treatment also depends on our ability to correctly diagnose dementia disorders in vivo. Currently, the clinical diagnosis of dementia diseases is sub-optimal, DLB in particular often goes unrecognized[8 9]. Accurate biomarkers and optimal clinical criteria are necessary to improve diagnostic accuracy.

1.3 Alzheimer`s disease

1.3.1 Epidemiology

AD is the most common cause of dementia, accounting for 60 - 80% of cases[10]. AD is very rare before middle age[11]. Age, a family history of AD and the apolipoprotein E (*APOE)* ε4 allele are the strongest risk factors for sporadic AD[12]. A population-based Dutch study found the cumulative incidence of AD to be 0.5% at 70 years, 5.7% at 80

years, 19.0% at 90 years and finally 25.0% in 100 years olds, which also constitute lifetime risk[13]. More women than men have AD; the main explanation being that more women survive to reach the age brackets with high AD prevalence[12]. Reported age-adjusted incidence rates are either the same for men and women or slightly higher for women[12].

1.3.2 Pathology

The pathology of AD is characterized by extensive synaptic and neuronal loss, and the hallmark lesions *neurofibrillary tangles* and *senile plaques*[14, 15]. The neurofibrillary tangles are intraneuronal lesions composed mainly of modified tau-protein[16]. Tau is a microtubule-associated protein involved in stabilization of microtubules in neuronal axons[15]. In AD, tau show abnormal phosphorylation of several amino acids, and the protein is sequestered in the neurofibrillary tangles[16].

Senile plaques are extracellular deposits of beta-amyloid (abeta)-peptides[14]. Amyloid precursor protein is a transmembrane protein that can either be cleaved by the α- and the γ-secretases, or by the β secretase and γ-secretase[7]. The latter pathway leads to formation of abeta-peptides of different lengths[7]. Some abeta peptides, including abeta42, are deposited in senile plaques[14], abeta42 is more fibrillogenic than the more common abeta40-peptide and associated with formation of abeta-deposits[15]. Soluble abeta42 oligomers might be toxic[17]. The morphology of senile plaques is diverse[14]. A subtype with abeta deposits at the center of a cluster of dystrophic neurites that sometimes show phospho-tau immunoreactivity is called *neuritic plaques*[14]. The pathogenesis of AD is still unknown. The so-called amyloid hypothesis stated that beta-amyloid dyshomeostatis is the key to initiating AD; this hypothesis is under debate with both supporters[17] and opponents[16]. Conformations of tau or abeta42 or both might spread in a seeding manner, similar to prion disorders[16]. Cell and animal models, as well as clinical, pathological and genetic studies, suggest that the etiology and pathogenesis of sporadic AD is heterogeneous with several pathways to the same clinical endpoint.

Six stages of neurofibrillary tangle pathology have been characterized by Braak and coworkers; “transentorhinal” (I-II), “limbic ”(III-IV) and “isocortical” (V-VI), with a mild and severe version of each stage[18]. Braak and coworkers postulated that the neurofibrillary tangle pathology starts in the transentorhinal cortex and then successively affects the rest of the limbic system prior to the neocortex. They could not find a similarly specific pattern in the development of senile plaques, as the early stages of amyloid deposits are heterogeneous[18]. Amyloid deposits were thus divided into only three stages; A, B and C. A was characterized by low densities of amyloid deposits in the neocortex, particularly the basal portions of the frontal, temporal and occipital lobes. B of medium densities of amyloid plaques in almost all neocortical association areas, the hippocampus is only mildly involved, and the transenthorhinal cortex might be affected[18]. In stage C almost all neocortical areas have high density of abeta-deposits, whereas the hippocampal formation, however, has relatively few deposits[18]. Adding to Braak and coworkers staging system for neurofibrillary tangle and amyloid-beta pathology, The Consortium to Establish A Registry for Alzheimer’s disease (CERAD) proposed a staging system for neuritic plaques in the neocortex, from C0: no neuritic plaques, C1: sparse, C2: moderate and C3 frequent plaques[19].

Beta-amyloid deposits have no clear correlation with cognitive impairment in AD dementia[20]. The tangle pathology, on the other hand, starts in regions of the brain vital for memory function[18], correlating to impaired memory being the first cognitive function to be affected in typical AD. Tangle pathology is robustly linked to cognition and neurodegeneration[18, 20, 21].

The senile plaques and neurofibrillary tangles are, per definition, present in all patients with AD, however, some degree of plaques and tangles are also found in a majority of 80 – 90-year-olds, including non-demented subjects coming to autopsy[16]. One study found that the presence of Braak tau stage V in persons with preserved cognition was associated with younger age, fewer neocortical neuritic plaques, and no comorbid neuropathological abnormalities, indicating that preserved cognitive function in the

presence of severe neurofibrillary tangle pathology is possible when the individual is younger and has no other pathology[22]. The same study also showed that when the interval between the last assessment of cognitive function and death was three years or more the number of people with apparent preserved cognitive function and severe AD pathology increased dramatically[22], however, when the interval between the last assessment and death increases so does the risk for undetected cognitive decline.

The Religious Order Study included Catholic nuns, priests and brothers from the USA without dementia, and followed them with annual cognitive assessments until death. Approximately 90% of persons meeting clinical AD criteria and 50% of mild cognitive impairment (MCI) patients met neuropathologically criteria for AD[23]. Data from this study were combined with results from another study of similar design[24]. Amongst the first 134 persons without dementia or mild cognitive impairment coming to autopsy, two had high likelihood and 48 intermediate likelihood of AD according to the 1997 National Institute on Aging-Reagan neuropathological AD criteria[25](the Criteria are described in section 1.5.2 "Neuropathological dementia diagnosis")[24]. These 50 patients scored significantly lower on episodic memory than the rest of the cohort[24]. In conclusion plaques and tangles occur in normal aging, but more severe AD pathology is more common in clinical AD – and pathology might represent prodromal AD.

1.3.3 Genetics

Genetically AD can be divided in two; rare, autosomal dominant forms with high penetrance that account for less than 1% of cases, and the multifactorial sporadic form with many associated genes[26]. The most common autosomal dominant forms are mutations in the genes for amyloid precursor protein, presenilin 1 and presenilin 2[26]. All three genes are closely linked to AD pathogenesis, amyloid precursor protein is the precursor to beta-amyloid and the presenilins have been shown to be the catalytic subunit of the γ-secretase necessary for abeta formation[11]. Many of the mutations in both the amyloid precursor protein and the presenilins are associated with increased

CSF abeta42/abeta40 ratio[27]. The autosomal dominant forms often cause early-onset AD, although most cases of early-onset AD are sporadic[26].

In the sporadic form genome-wide association studies have found multiple risk genes. Some related to beta-amyloid processing, but also genes that code for proteins involved in the immune system, synaptic functioning, the cytoskeleton, and axonal transport, lipid metabolism, regulation of gene expression and posttranslational modification[26]. However, the strongest genetic risk factor in sporadic AD is the ε4 allele of the *APOE*-gene[26]. The brain is the most cholesterol-rich organ in the body[28]. Apolipoprotein E is involved in cholesterol transport, and a regulator of lipoprotein metabolism with effects also on inflammation and neuroplasticity[28, 29]. Research suggests a link between Apolipoprotein E and amyloid pathology, Apolipoprotein E having been found to have an effect on the clearance of abeta aggregations[29]. Of the three alleles of the *APOE* gene, the ε3 is most frequent and considered neutral regarding risk for AD. ε2 has the lowest frequency and is associated with decreased risk for AD and later age at onset[28, 29]. The ε4 allele is associated with increased neuropathological abeta aggregates, and significantly lowers the age of AD onset[29]. AD risk is three times higher in ε4 heterozygotes than non-carriers, and 12-15 times higher in homozygotes[27, 29]. The allele is carried by > 50 % of AD patients while the allele frequency in the population is only 15%[28].

1.3.4 Clinical features

Cognitive symptoms

The neuropathological AD process starts many years before the gradual onset of cognitive symptom. In addition to cognitive decline, the diagnosis of dementia, according to the ICD-10, and major neurocognitive disorder, the Diagnostic and Statistical Manual of Mental Disorders (DSM) 5 equivalent, also requires impairment of functions of daily living. This requirement that the cognitive decline impairs function of daily living is of vital clinical importance, but at the same time also somewhat subjective as it depends on the individual`s normal activity level which

might vary from a demanding job to a sedentary retired life. Studying early disease and addressing patients with cognitive impairment that falls short of dementia has paved the way for the construct of "mild cognitive impairment", MCI. MCI can be defined as either reports of cognitive impairment from patient or carer that can be verified on cognitive tests or evidence of cognitive decline from previous level of functioning in persons that are independent in activities of daily living[30], however, several other definitions exist. The MCI population is heterogeneous[1], and from MCI there are three different trajectories; some remain stable, some improve and some develop dementia. The risk of developing dementia is higher in a person diagnosed with MCI compared to the general population[1]. MCI patients with prodromal AD typically have impairment of episodic memory[31]. The National Institute on Aging and the Alzheimer´s Association have jointly published criteria for MCI due to AD, including research criteria where AD biomarkers are used for the diagnosis of MCI due to AD (for more information on AD biomarkers see section 1.3.5)[31]. Based on the biomarker supported criteria, 46% of MCI patients had a high likelihood of prodromal AD (both beta-amyloid and neuronal injury biomarker positive) with a three year conversion rate to AD of 59%[32]. In comparison, 22% of individuals with a positive beta-amyloid but negative neuronal injury biomarker, and 24% of individuals with a negative beta-amyloid but positive neuronal injury biomarker progressed to AD over three years, and only 5% of individuals with low likelihood of MCI due to AD (negative abeta42 and neuronal injury biomarkers) developed AD[32].

The typical presentation of AD is the amnestic syndrome, with impairment on delayed recall tests not significantly improved with cuing[33]. Impairment of executive functions, such as the ability to plan and execute goal-directed behavior is also common[11]. Approximately 6 – 14 % of cases have an atypical presentation, with relatively preserved memory at disease debut[33]. Atypical variants usually have an earlier onset than the amnestic variant[33]. It has been suggested that atypical variants can be divided into specific subtypes. A *logopenic variant* with early impairment of retrieval of single words and repetition of sentences[33]. A *frontal variant* with early and predominant behavioral changes or predominant executive dysfunction on neuropsychological

testing[33]. A *posterior variant,* which is divided into an occipitotemporal and a biparietal variant respectively, with the occipitotemporal variant dominated by impairment of visuoperceptive functions such as identification of objects, faces, and symbols and the biparietal of early impairment of visuospatial function, limb apraxia or neglect[33].

After the emergence of the first symptoms, the neurodegeneration progresses, leading to further deterioration of cognitive and ADL functions, leading to loss of independent functioning. The patients often first require assistance at home, and subsequently, nursing home placement is often needed. In very severe AD the patient can no longer walk, talk or eat independently. The time to progression from diagnosis of AD to severe dementia is highly heterogeneous. A population-based incidence study found that 30 - 58% of AD patients progressed less than one point per year on the Mini-Mental State Evaluation (MMSE) even 5-7 years after the onset of dementia, women and younger patients progressed faster[34]. Reported time from diagnosis to death and loss of life years vary considerably between studies[35]. A German incidence study found a median survival of 3.2 years after AD diagnosis in a population of persons > 75 years of age, mean age of onset was 85 years[36]. A population-based study of incident dementia in England and Wales found median survival of 4.5 years after estimated dementia onset, survival varied from median 10.7 years in those <70 years to 3.8 in individuals > 90[37].

Non-cognitive symptoms

Non-cognitive symptoms such as motor-, neuropsychiatric- and behavioral symptoms are common in the course of AD[38]. 50% of all-cause MCI and 80% of all cause-dementia experience at least one neuropsychiatric symptom from the onset of cognitive impairment[39]. Neuropsychiatric symptoms include apathy, depressive symptoms, anxiety, irritability and aggression, non-aggressive agitation, hallucinations, delusions, misidentification, apathy, sleep-problems, wandering and elation[40]. A systematic review found a higher prevalence of these symptoms in studies of patients with moderate to moderately severe dementia than in studies including patients with severe dementia only[40]. In addition to prevalence, the persistence of

neuropsychiatric symptoms over time is important for the impact on patients and carers. Hyperactivity and apathy have both high incidence and persistentence, anxiety and depression have moderate incidence and low persistence, while psychotic symptoms have moderate to low incidence and low persistence[40].

Neuropsychiatric and behavioral symptoms are associated with increased caregiver burden, psychological distress, and depression[41]. These symptoms might also increase the risk of nursing home admission[41] and predict the development of dementia from mild cognitive impairment[42]. They are also associated with faster progression to severe AD[43] and shorter survival[43, 44]. Although the field has received increased research interest in the later years, the pathology underlying the highly variable development of neuropsychiatric symptoms in individuals with AD is largely unknown[45-55].

1.3.5 Biomarkers

The World Federation of Societies on Biological Psychiatry´s Task Force on Biological Markers summarized previously published criteria for diagnostic biomarkers in neurodegenerative dementias in general and AD in particular. A marker should be;

- linked to fundamental features of the neuropathology,
- validated in neuropathologically confirmed cases,
- able to detect the disease early in its course and distinguish it from other dementias,
- non-invasive, simple to use and inexpensive,
- not influenced by symptomatic drug treatment[7].

CSF and blood-based biomarkers

The most commonly used and promising AD biomarker modalities will be described in brief here. The abeta42 peptide and the proteins t-tau and p-tau, are the most common CSF biomarkers. Most p-tau assays target tau phosphorylated at amino acid

181, p-tau181. In AD abeta42 is decreased and t-tau and p-tau increased compared to healthy controls[56]. The CSF markers are closely linked to AD pathogenesis. The abeta42 decrease likely represents its deposition in amyloid plaques, t-tau is a marker of the intensity of neurodegeneration and p-tau is the modified tau form sequestered in neurofibrillary tangles[56]. The markers have been reported to identify AD at the mild cognitive impairment stage with sensitivities and specificities around 85-90%[11]. As mentioned previously, AD neuropathological lesions can also be found in people without clinical AD dementia at the time of death, and multiple pathologies are commonly present in dementia patients. Thus biomarkers that detect neuropathological AD lesions will never predict clinical AD dementia perfectly. However, a normal biomarker profile has been shown to have high negative predictive value of AD development in MCI[11]. Blood-based biomarkers would be attractive but have proved difficult to find. No blood-based markers are included in current research criteria for AD[33, 57].

Imaging- and nuclear medicine biomarkers

Atrophy of the medial temporal lobe on MRI is an AD biomarker, hippocampal volumetry might be superior to visual rating[11]. Sensitivity and specificity for medial temporal lobe atrophy on MRI across assessment methods is 75% and 81% for AD dementia vs normal controls, and 62% and 73% respectively for MCI progressing to AD vs non-progressive MCI[58].

Both ^{18}F-fluorodeoxyglucose (FDG) PET and PET with tracers that bind to core AD pathology are used. The FDG PET measures glucose uptake by glial cells and neurons, with the finding of a specific pattern of hypometabolism in the temporoparietal and posterior cingulate regions being suggestive for AD[11]. Temporoparietal hypometabolism has a sensitivity of 86% and specificity of 84% for AD dementia vs controls and sensitivity of 76% and a specificity of 74% for MCI progressing to AD vs non-progressive MCI[58]. Specific for AD neuropathological lesions, amyloid tracers were first developed more than a decade ago, while tau tracers are a more recent addition[20]. The ^{11}C-PiB (PiB) tracer binds to abeta in plaques and other lesions, and the pattern of binding is similar to the pattern of amyloid plaques found at autopsy[20].

Increased PiB binding correlates strongly with decreased abeta42 in CSF[20]. As ^{11}C-PiB has a 20-minute half-life, use is limited to centers with a cyclotron, and this has inspired the development of tracers labeled with fluorine-18 (^{18}F) which has 110 minutes half-life[20]. Different ^{18}F tracers appear to replicate the PiB results[20].

Sensitivity for amyloid imaging with PET is 88% for AD against healthy aging, and 82% for MCI progressing to AD vs stable MCI, with specificities of 85% and 56% respectively[58]. The low specificity for progressive vs non-progressive MCI might in part be due to amyloid accumulations preceding dementia by many years. Amyloid deposits have been found in cognitively intact individuals, perhaps representing pre-clinical disease[20]. Currently this is a limitation for the use of amyloid imaging as an AD biomarker.

The sequential spreading of tau- and neurofibrillary tangle pathology[18] and its correlation with cognitive impairment and neurodegeneration[14, 20] make tracers that bind to this pathology attractive as AD markers. Several tracers have been developed, such as THK5317, THK5351, AV-1451 and PBB3[59]. Tau tracer could be used for diagnostic purposes, as well as staging and monitoring of disease progression as it provides the opportunity to follow the pattern of tau depositions in vivo[59]. However, much research is still needed to ascertain the binding properties of the tracers to different tau aggregates and validation of their abilities as biomarkers[59].

1.4 Lewy body disease with dementia

1.4.1 Clinical diagnostic criteria

DLB

The ICD-10 does not include criteria for DLB, and in the DSM DLB was first introduced with the publication of DSM 5 in 2013 as Major and Minor Neurocognitive disorder with Lewy bodies. In research, the criteria published by the DLB consortium by McKeith et al are most commonly used. The first criteria were published in 1996,

and have since been revised twice[60-62], for details see table 1. The central feature of dementia has been consistent across revisions, as have the core features fluctuating cognition, visual hallucinations and parkinsonism. However, the required severity of parkinsonism has been revised. In the 1996 criteria parkinsonism was described as typically mild[60], in the 2005 as equally severe to age-matched PD patients[61] and in the 2017 criteria, only one of the cardinal features of PD is necessary to fulfill the parkinsonism trait[62].

The 2005 criteria added "suggestive features"; REM-sleep behavior disorder (RBD), severe neuroleptic sensitivity and evidence of reduced dopamine uptake in the basal ganglia demonstrated by SPECT or PET, with one core and one suggestive feature sufficient for a probable DLB diagnosis[61]. In 2017 the "suggestive features" category was removed, but replaced by "indicative" and "supportive biomarker" categories. Reduced dopamine uptake in the basal ganglia by PET or SPECT (dopamine transporter imaging), abnormal MIBG myocardial scintigraphy and polysomnography confirming REM sleep without atonia were now defined as an indicative biomarkers and RBD included as a core feature[62]. One core feature and one indicative biomarker are now sufficient for a diagnosis of probable DLB. All three sets of criteria have addressed the distinction between DLB and PDD. Dementia developed in established PD should be labeled PDD. Dementia with subsequent parkinsonism and dementia and parkinsonism emerging concomitantly should be labeled DLB. In research settings the one-year-rule is recommended, if dementia develops within a year after onset of parkinsonism the diagnosis should be DLB.

Table 1: Criteria for clinical diagnosis of probable and possible DLB by the DLB consortium

	1996[60]	2005[61]	2017[62]
Central feature	"A dementia syndrome with sufficient severity to affect normal functioning. Memory is not always affected in the early stages, but deficits on tests of attention, visuoperception and executive functions might be more severe and occur early."		
Core features	"Fluctuating cognition with pronounced variations in attention and alertness."		
	"Visual hallucinations that are typically well formed and detailed."		
	"Spontaneous features of parkinsonism, typically mild. Rigidity and bradykinesia are the usual extrapyramidal symptoms."	Spontaneous parkinsonism. "The severity of extrapyramidal motor features in DLB is generally similar to that of age matched patients with PD (...). Rest tremor is less common."	Spontaneous parkinsonism. Requires only one of the cardinal features of PD; bradykinesia, rest tremor or rigidity.
	-	-	RBD which may precede cognitive decline.
Suggestive features	-	RBD, severe neuroleptic sensitivity, positive DAT imaging.	-
Supportive features	"Repeated falls, syncope, transient loss of consciousness, neuroleptic sensitivity, systematized delusions, hallucinations in other modalities"	As 1996 + severe autonomic dysfunction, depression, no medial temporal lobe atrophy, positive perfusion SPECT/PET, positive MIBG myocardial scintigraphy, typical EEG.	As 1996 and 2005, the only difference is that more features are listed such as hyposmia, postural instability, apathy, hypersomnia and anxiety.
Indicative biomarkers	-	-	Positive DAT imaging. Positive MIBG myocardial scintigraphy, Polysomnographic confirmation of RBD.
Supportive biomarkers	-	-	No temporal lobe atrophy, positive perfusion SPECT/PET, typical EEG.

A diagnosis of DLB is less likely	"In the presence of cerebrovascular disease evident as focal neurologic signs or on brain imaging."		"In the presence of any other physical illness or brain disorder including cerebrovascular disease, sufficient to account in part or in total for the clinical picture, although these do not exclude a DLB diagnosis and may serve to indicate mixed or multiple pathologies contributing to the clinical presentation,..."
	"In the presence of any other physical illness or brain disorder including cerebrovascular disease, sufficient to account in part or in total for the clinical picture."		
	-	"If parkinsonism only appears for the first time at the stage of severe dementia"	"If parkinsonian features are the only core clinical feature and appear for the first time at a stage of severe dementia"
Probable DLB, at least:	Two core features.	Two core features or one core and one suggestive feature.	Two core features, or one core feature and one indicative biomarker.
Possible DLB:	One core feature.	One core or one suggestive feature.	One core feature or one indicative biomarker.
PDD vs DLB	<12 months of parkinsonism prior to dementia → DLB >12 months of parkinsonism prior to dementia → PDD		

Abbreviations: **RBD:** REM sleep behavior disorder. **No temporal lobe atrophy:** "relative preservation of medial temporal lobe structures on CT/MRI scan"[62], **Positive perfusion SPECT/PET:** "generalized low uptake on SPECT/PET perfusion/metabolism scan with reduced occipital activity ± the cingulate island sign"[62]. **Typical EEG:** 2005: "prominent slow wave activity on EEG with temporal lobe transient sharp waves"[61]. 2017: prominent posterior slow-wave activity on EEG with periodic fluctuations in the pre-alpha/theta range[62]. **Positive DAT imaging:** "Reduced dopamine uptake in basal ganglia demonstrated by SPECT or PET"[62]. **Positive MIBG scintigraphy**: "Abnormal (low uptake) on ^{123}iodine-MIBG myocardial scintigraphy"[62].

PDD

Although clinically defined as a movement disorder, the cognitive impairment in PD is common and developing gradually. Motor impairment also influences function, thus pinpointing the time an individual reaches dementia can be difficult. The ICD-10 defines PDD simply as "dementia developing in the course of established Parkinson disease. No particular distinguishing clinical features have yet been demonstrated"[4]. The International Parkinson and Movement Disorder Society has published PDD criteria, requirements for probable PDD are[63];

- Dementia, defined similarly to the ICD-10 dementia criteria, but without the specification that memory must be affected.
- A cognitive profile with impairment of at least two of the following domains; executive functions, visuospatial functions, attention, and free recall – which usually improve with cueing.
- Behavioural symptoms such as hallucinations, apathy, delusions, depression, anxiety, and daytime hypersomnolence support the diagnosis but is not mandatory.
- Other pathology associated with cognitive impairment, but judged not to be the cause of dementia, should not be present, for instance, significant vascular lesions. The time between the onset of motor and cognitive symptoms should be known.
- There should not be sufficient vascular pathology to fulfil criteria for vascular dementia, major depression or other reasons which might in itself explain the cognitive impairment.

1.4.2 Epidemiology

DLB

The exact incidence and prevalence of DLB are unknown for several reasons. Population-based incidence studies that break dementia into different diagnosis are few, as they are expensive and difficult to organize. The 1996, 2005 and 2017 DLB criteria might not identify the same patients, and DLB is probably underdiagnosed[64].

A systematic review found that in the studies published after 2005, the prevalence of DLB in the population > 65 years was 0 – 1.2%, and between 0 – 9.7% of dementia cases had DLB[64]. Average incidence was found to be 0.87 cases / 1000 person-years in the population > 65[64]. This estimate was based on three studies; one applied the 1996 criteria and reported an incidence of 0.57 /1000 person-years, and two studies using the 2005 criteria found 1.12 and 1.40 /1000 person-years respectively[64]. In secondary care settings, the mean prevalence of DLB, usually reported as the proportion of

dementia with DLB, was 7.5%[64]. Prevalence varied greatly from 2.2% - 24.7% of cases, again the studies using the 2005 criteria reported the highest prevalence[64]. There were no clear differences in gender, but a trend towards positive correlation between age and DLB prevalence[64].

PDD

PD prevalence increases gradually with age, from 0.11% in individuals aged 50 to 59 years, to 0.43 in individuals 60 -69, 1.1% in individuals aged 70- 70 years and 1.9% in individuals > 80 years[65]. PD incidence is higher in men than in women[66]. Cognitive impairment is common in PD, at time of diagnosis prior to treatment approximately 1 in 5 patients has mild cognitive impairment[67]. The point prevalence of dementia in PD is approximately 30%[68] and increases with disease duration. In an incidence cohort, the prevalence of dementia was 46% in survivors after 10 years[69]. A longitudinal study found that 75% of PD patients were diagnosed with dementia prior to death, while 9% died early from unrelated causes and another 11% died more than one year after their last cognitive assessment[70]. The same study found a cumulative prevalence of dementia of 83% in the few who survived 20 years after PD diagnosis[70].

A review of 18 longitudinal studies found that the three most influential factors for cognitive impairment and dementia in PD are hallucinations, older age, and severity of PD symptoms[71]. Impaired speech, PD onset at a higher age, the severity of bradykinesia and axial impairment (i.e. impairment of gait and postural instability), shorter education, depression, and male gender are also risk factors[71]. In addition to this, RBD[72], orthostatic hypotension[72], mild cognitive impairment[72, 73] and reduced abeta42 in CSF[73, 74] have been found to predict the development of dementia in PD.

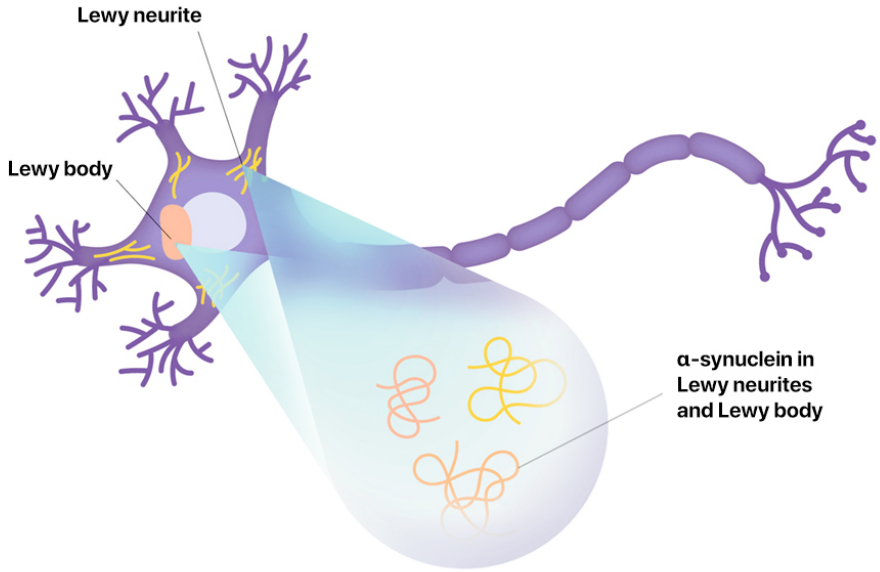
1.4.3 Pathology

α-synuclein

The hallmark features of LBD are Lewy bodies in the cell soma and Lewy neurites in neuronal cell processes, and misfolded α-synuclein is the main constituent of both[16]. α-synuclein is a major part of the central nervous system proteome and is also expressed

outside the central nervous system[75]. The function has yet to be thoroughly described, but several reports point to a role in synaptic vesicular transport[75]. There is no evidence for a difference between PD and DLB at the protein level.

Figure 1: Lewy bodies and Lewy neurites in neuron



In addition to Lewy body diseases DLB and PD, multiple system atrophy (MSA) is also associated with α-synuclein aggregations. In MSA these lesions occur predominantly in oligodendroglia and Schwann cells, as opposed to neurons like Lewy bodies and neurites[76]. The Lewy body pathology is associated with neuronal loss, but whether the Lewy bodies and Lewy neurites are neurotoxic is unknown[77]. The toxic agent might be α-synuclein oligomers (i.e. several α-synuclein proteins bound to each other), α-synuclein fibrils (i.e. much larger structured aggregates)[75, 76] or another substance. The observation that embryonal neural tissues transplanted into the striatum of PD patients developed α-synuclein pathology lead to a theory that α-synculein is a prion-like protein[75, 76]. Mitochondrial dysfunction seems to play a key role in PD[78]. Concomitant amyloid plaques and neurofibrillary tangles are common in LBD[5, 79], vascular lesions are also frequently found[5].

DLB

The 1996 criteria by the DLB consortium divided neuropathological Lewy body pathology into brainstem predominant, limbic (transitional) and neocortical, based on semiquantitative assessments of distribution and frequency[60]. These criteria required only the presence of Lewy bodies somewhere in the brain for a patient with dementia to be classified as DLB, regardless of the presence of other pathology[60]. Thus up to 60% of AD patients would be neuropathologically classified as DLB[61]. The 2005 criteria addressed this issue by including likelihoods of the clinical DLB syndrome with different combinations of Lewy body and AD pathology, combinations could have “low”, “intermediate” or “high” likelihoods of clinical DLB[61].

The criteria from 2017 are presented in table 2, reproduced from the original publication. In the 2017 criteria[62], the neuropathological AD criteria were updated to the most recent National Institute on Aging-Alzheimer´s Association guidelines[80] and amygdala predominant and olfactory bulb only stages of DLB pathology were added. Both are considered low likelihood for the DLB syndrome, but possibly represent a prodromal stage of disease[62]. The new criteria also include a sub-classification with regards to atrophy of the substantia nigra and corresponding likelihood for parkinsonism[62].

Table 2: Assessment of the likelihood that pathology findings are associated with a typical, dementia with Lewy bodies, clinical syndrome

Alzheimer disease neuropathologic change	NIA-AA None/low (Braak stage 0-II)	NIA-AA Intermediate (Braak stage III-IV)	NIA-AA High (Braak stage V-VI)
Lewy-related pathology			
Diffuse neocortical	High	High	Intermediate
Limbic (transitional)	High	Intermediate	Low
Brainstem-predominant	Low	Low	Low
Amygdala-predominant	Low	Low	Low
Olfactory bulb only	Low	Low	Low
Substantia nigra neuronal loss to be assessed (as none, mild, moderate, and severe) in order to subclassify cases into those likely or not to have parkinsonism.			

Abbreviation: NIA-AA = National Institute on Aging-Alzheimer's Association guidelines for the neuropathological assessment of Alzheimer diseases[80]. The table is reproduced with permission from Mc Keith et al[62].

As evident from the table, the likelihood of a clinical DLB syndrome increases with limbic and ultimately neocortical Lewy related pathology, and concomitant AD pathology decreases the likelihood for a DLB phenotype.

PDD

At its core, Parkinson`s disease is a clinical syndrome with degeneration of dopaminergic neurons in the substantia nigra pars compacta. The gold standard of diagnosis has been neuropathological verification of this degeneration and the presence of Lewy related pathology[81]. Braak and coworkers have proposed a 6 step caudal-to-rostral spreading of Lewy related pathology in PD; stage 1 where the pathology starts in medulla oblongata and frequently also the anterior olfactory nucleus, stage 2 where pathology spreads in the medulla oblongata and starts to affect pontine structures, stage 3 with involvement of mesencephalon including substantia nigra pars compacta, stage 4 were pathology involves more limbic structures and temporal cortex, and stage 5 and 6 where neocortical regions are involved[82]. This theory could explain the temporal development of clinical PD; the first symptoms are frequently non-motor and include obstipation, RBD and olfactory dysfunction[83] which

could be explained by stage 1 and 2. The subsequent development of the motor symptoms corresponds to stage 3. Stages 4 – 6 would be consistent with the many non-motor symptoms of advanced PD, including dementia[82].

Not all autopsy studies support this theory, however the correlation between the severity of cortical Lewy body pathology and dementia is strong[81]. It is important to remember that Lewy body pathology is not the sole underlying pathology of cognitive impairment in PD. Neurofibrillary tangles and amyloid plaques are common, and severe concomitant AD pathology is strongly associated with dementia[5, 79, 84, 85]. A higher number of cerebrovascular lesions have been found in PDD patients compared to PD[86]. DLB and PDD cannot be separated neuropathologically. Thus, in summary, cortical Lewy body pathology is strongly linked to cognitive impairment. Comorbid AD pathology is common and likely affects the clinical presentation – both the presence of the clinical DLB phenotype and the risk of dementia in PD.

1.4.4 Genetics

DLB

Increased risk for DLB and visual hallucinations have been found in siblings of DLB patients compared to siblings of AD patients, odds ratio (OR) 2.3 and 2.3 respectively[87]. The largest genome-wide association study performed to date point to three different loci being strongly associated with DLB risk[88]. First, like in AD, the *APOE*-gene is involved, and carriers of the *APOE* ε4 allele were reported to have OR of 2.4 compared to non-carriers[88]. Interestingly carriers of an *APOE* ε2 allele were by another study reported to have decreased risk for DLB, with an OR of 0.4[89]. The second loci found to have a significant association with DLB risk is the gene encoding glucocerebrosidase (*GBA*), a lysosomal enzyme which may influence α-synuclein processing[90]. The effect of this loci was an OR of 2.6 for DLB compared to controls[88]. The third loci found to be strongly associated with DLB risk was the *SNCA* gene coding for α-synuclein, with an OR of 0.7, translating to a reduced risk of DLB of variants compared to controls[88]. Highly penetrant mutations in genes known to cause

monogenetic forms of neurodegenerative diseases have been found in 4.4% of patients with DLB[91]. The hereditability of DLB was estimated to 36%[88].

PDD

Approximately 15 % of PD patients have a family member with PD, and 5-10% of cases are caused by either autosomal dominant or autosomal recessive mutations in single genes[92]. 10 genes with autosomal dominant mutations have been identified, including in the *SNCA* and leucine rich repeat kinase 2 (*LRRK2*)genes, mutations in the LRRK2 is the most common genetic cause of PD[92]. The LRRK2 protein is involved in the cytoskeleton, vesicular transport, protein synthesis, and lysosomal systems[92]. Mutations may lead to degeneration and death of dopaminergic neurons[92]. The genes that code for proteins DJ-1 and parkin are among the 9 autosomal recessive genes identified to date[92]. Both proteins are vital for mitochondrial function, and mutations cause early onset PD[81, 92]. Genetic risk factors for PD include mutations in the *GBA*, *LRRK2* and *SCNA* genes, and in the *MAPT* gene coding for the protein tau (see the AD section)[92]. Interestingly, genetic risk factors for cognitive impairmentand dementia in PD have been discovered, including the *APOE* ε4 allele[93, 94], and specific mutations in the SNCA[93], GBA[95] and MAPT[94] genes.

1.4.5 Clinical features

Prodromal phase

Interviews with LBD patients at the time of diagnosis indicate that the first symptoms 10 – 15 years prior to diagnosis are non-cognitive[96]; decreased sense of smell, RBD, constipation, dizziness while standing, urinary incontinence and increased salivation and sweating[96]. Subsequently, some experience delirium, depression and psychosis, and later cognitive impairment of a non-amnestic subtype, visual hallucinations, illusions, misconceptions, and parkinsonism occur[96]. Whether prodromal DLB and PD can be differentiated from each other is a priority but not yet known[96]. Although this approach is susceptible to recall bias, and many of these symptoms common in normal aging, these findings suggest that prodromal DLB might be heterogeneous with both autonomic, psychiatric and cognitive features.

Idiopathic RBD is an important prodrome for LBD[97]. After a follow-up of mean 3.6 years, 17 of 76 idiopathic RBD patients developed PD, 15 DLB, and two MSA[98]. Although most MCI who progress to dementia have AD, some will also develop DLB. MCI patients who later developed DLB (DLB-MCI) scored worse on visuospatial function- and letter fluency tests compared to stable MCI and MCI patients who developed AD (MCI-AD), however scored better on tests of episodic memory than MCI-AD[99]. The MCI-DLB group had higher UPDRS-III scores and more fluctuating cognition and RBD than the stable MCI and MCI-AD patients[99].

Cognitive profiles

DLB patients on average have a profile of cognitive impairment dominated by impaired attention, executive- and visuospatial functions rather than impairment of memory and naming[62, 100]. In the Demvest-cohort mild DLB patients scored significantly worse than mild AD patients on visuoconstruction (MMSE pentagon test) and all tests involving attention and executive functions, with the exception of verbal fluency[100]. The AD patients scored significantly lower on memory tests, both delayed recall and recognition[100]. Visuoperception was similar in both groups but was assessed with a test which required patients to name objects and animals[101]. Naming can be more difficult for AD patients resulting in findings that are difficult to interpret[100]. The cognitive profile was able to correctly classify 79.1% of patients, however, 32% of the DLB patients were misclassified[100], thus there is a significant overlap between cognitive profiles in early AD and DLB.

The cognitive profile in PDD includes impairment of executive functions, attention, and visuospatial functions, but not always delayed recall at dementia onset[63]. Whilst few studies have compared DLB and PDD with equal severity of dementia, some have found DLB patients to have more impaired attention, others found no difference[102]. Both DLB and PDD have been found to have a higher likelihood of a “subcortical” cognitive profile, i.e. impairment of initiation, construction and attention vs the “cortical” profile common in AD, with severe impairment of delayed memory, but more preserved attention, initiation, and construction[102, 103].

Non-cognitive symptoms

Fluctuations

The main clinical features that characterize DLB and separate it from other dementias are increased prevalence of cognitive fluctuations, visual hallucinations, parkinsonism, and RBD compared to other dementing disorders. Fluctuations in cognitive function and attention are perhaps the core DLB feature that is most difficult to assess clinically. Fluctuations can be rapid, lasting minutes to hours or slower – with weekly to monthly variations[60]. They can be experienced by carers as periods of daytime drowsiness, episodes of disorganized speech or staring into space[62] or as the patient being "switched off"[60]. Sometimes fluctuations may be as extreme as transient episodes of non-responsiveness and therefore difficult to differentiate from syncope[62].

However, fluctuations are not specific for DLB. Fluctuations in both motor- and non-motor symptoms are also common in advanced PD and may be related to long-term dopaminergic treatment[81]. One study which tested alertness and cognition during 48 hours of observation found greater fluctuations of cognition in DLB compared to PD patients without dementia, but no difference in fluctuations in alertness[104]. Fluctuations also become increasingly common in more advanced dementia of other etiologies[105] and are thus less specific for DLB in moderate and severe dementia[8]. Repeated tests of cognition and attention, computerized or manually administered can be used to detect fluctuations[104, 106] but structured scales administered to carers are the most used tools for assessment[107, 108]. The use of one such measure of fluctuations in the diagnostic process is advised by the DLB consortium[62].

Visual hallucinations

Early visual hallucinations in the course of dementia should lead to a suspicion of DLB; visual hallucinations are reported in 35% - 85% of autopsy-confirmed cases[109]. Hallucinations are typically well-formed; patients typically see people or animals[62]. Some, but not all, have preserved insight that these experiences are illusions, and the emotional content of the hallucinations is variable[62]. However, visual hallucinations also occur in other age-related disorders. Significant eye disease increases the risk of

visual hallucinations, which are usually simple, but complex hallucinations may also occur[110]. Visual hallucinations are also common in AD, but usually present in the more advanced stages compared with DLB[109]. In an autopsy verified study most visual hallucinations in DLB occurred during the first 5 years after estimated dementia onset[109]. The presence of visual hallucinations during in this period were associated with high likelihood of autopsy verified DLB vs AD[109]. Hallucinations in other modalities also occur in DLB, but less frequently[62].

Both illusions, hallucinations and delusions occur in PD, with the more severe forms being referred to as "PD psychosis". Typically the onset is insidious, but with increasing severity of PD, fully formed visual hallucinations can evolve[111]. Insight is usually preserved in early stages but often diminishes with disease progression. Loss of insight is associated with impaired cognition[111]. Delusions and non-visual hallucinations, such as tactile, olfactory and auditory hallucinations, might also develop as PD progresses[111]. PD psychosis is common, in very early PD 42% of patients reported minor phenomena, and overall 22 – 38% of PD patients have complex visual hallucinations[112, 113]. Visual hallucinations are linked to limbic Lewy body pathology, but reports have also implicated tau and amyloid pathology in the frontal, parietal and hippocampal areas and the cholinergic system[111]. Dopaminergic treatment increases the risk of psychosis, although to what extent, and the exact nature of the effect is still controversial[111].

Parkinsonism in DLB

Spontaneous features of parkinsonism are common in DLB[62]. One study found parkinsonism within 3 years of disease onset in 82% of autopsy verified DLB[114]. Parkinsonism in DLB has been characterized more by postural instability, gait difficulty and facial immobilization and less rest tremor than PD[61]. The 2017 revision of the DLB criteria acknowledged that parkinsonism in many DLB patients falls short of the International Parkinson Disease and Movement Disorder Society PD criteria, i.e. bradykinesia in addition to rest tremor, rigidity or both[115]. Thus the 2017 criteria require only one of the cardinal PD criteria to fulfill this criterion[62]. However, the need for careful evaluation to avoid misidentification due to inability to complete

neurological examination tests due to cognitive impairment or comorbid conditions is stressed[62].

REM sleep behavior disorder

The inclusion of RBD as a core criterion in DLB has been shown to increase the diagnostic accuracy of clinical DLB diagnosis in autopsy verified cases[116]. Polysomnography is used to detect RBD, this is, however, costly and subjects must be able to and willing to cooperate[117]. The Mayo Sleep Questionnaire, where the bed partner is asked about dream enactment behavior, has in some studies been used instead of polysomnography, but requires a bed partner or someone who regularly sees the patient sleep[117]. Polysomnography is needed when there is doubt as to whether the sleep disturbance is RBD or other conditions common in old age, such as obstructive sleep apnea, confusional awakening, and periodic leg movement[62].

Other symptoms

Autonomic dysfunction in LBD leads to symptoms such as constipation, orthostatic hypotension, and urinary incontinence[62, 81]. Patients with DLB often have adverse reactions to antipsychotic agents, from mild symptoms such as drowsiness to irreversible cognitive decline, worsening of parkinsonism, and full-blown malignant neuroleptic malignant syndrome[118]. In PD, antipsychotics other than clozapine, quetiapine, and pimavanserin frequently worsen parkinsonism[81]. Excessive daytime sleepiness, increased risk for falls, anxiety, depression, apathy, and hyposmia have also been found to be associated with LBD[62, 83, 119].

Prognosis

Several previous studies have found different trajectories after diagnosis in AD and DLB[120, 121]. The largest study published to date with more than 800 participants found a faster annual decline in MMSE scores in DLB (2.1) compared AD (1.6) and PDD (1.8) from mild dementia, after adjusting for MMSE at inclusion[120]. In the Demvest study, DLB patients had more rapid cognitive decline than the AD patients, with DLB patients progressing to severe dementia 5 months earlier than AD patients[121]. Contrary to this, a review of 6 small studies found no difference in the rate of cognitive decline

between AD and DLB[122]. However, patients with mixed DLB and AD pathology may have the most rapid decline[123].

Admission to residential care is an important milestone of disease progression; an indicator for large care needs and associated with high costs. Known triggers for nursing home placement in dementia include neuropsychiatric symptoms such as psychosis and aggression, as well as motor impairment leading to greater disability[123]. Thus DLB patients might require early nursing home placement due to the complex clinical profile including neuropsychiatric symptoms[123]. In the Demvest study, median time from mild dementia to nursing home placement was almost two years shorter in DLB than for the AD patients[124]. Others studies, however, have failed to find a difference[123]. Three of four studies that compared financial burdens in AD and DLB found higher costs in DLB[123]. Comorbid AD pathology, the *APOE* ε4 allele, gait abnormalities, fluctuating cognition, and hallucinations are associated with shorter survival in DLB[123]. A study with patients diagnosed in clinical practice in the United Kingdom found a median survival after diagnosis of 3.7 years in DLB compared to 7.0 years in the AD group[125], similar differences was found in the Demvest cohort[126]. These findings of shorter survival in DLB compared to AD are backed by a majority of the relatively few studies published[127].

MCI is a prodrome for dementia in PD. However, as in the normal population, MCI may revert to normal cognition, thus the presence of MCI does not necessarily herald dementia[112]. It has been suggested that MCI in PD is in fact two different syndromes; one fronto—subcortical syndrome with executive deficits associated with loss of dopaminergic innervation to the striatum, and a posterior cortical syndrome with impairment of visuospatial functions and memory associated with Lewy body pathology and cholinergic deficits[94]. Research suggests that the cortical posterior syndrome is a stronger risk factor for dementia[112].

The cumulative prevalence of dementia is very high in PD – but the timing from PD onset to cognitive impairment and dementia varies markedly[128]. After diagnosis, most

patients experience a period with very little or no cognitive decline, followed by an inclination point leading to more rapid cognitive decline[128]. Whilst the time to this inclination point is highly variable, the prognosis after the onset of dementia is more homogenous[128]. In addition to dementia, visual hallucinations, regular falls, and need for residential care and death are important milestones in PD. Time from diagnosis to the first milestone is significantly longer in younger patients[129]. After the patient reaches the first milestone, prognoses are more similar regardless of age[129].

1.4.6 Biomarkers

CSF biomarkers

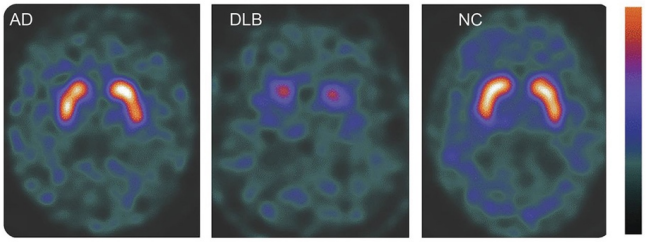
The common occurrence of AD pathology in DLB also affects the interpretation of the CSF AD biomarkers abeta42, t-tau, and p-tau. A CSF AD profile has been found in 25% of DLB patients[130], and the presence of reduced levels of abeta42 are associated with more rapid cognitive decline in clinically diagnosed DLB patients[131]. As mentioned previously, reduced abeta42 in early PD has consistently been shown to predict more rapid cognitive decline in early PD[73, 74, 132].

Biomarkers that could reliably detect α-synuclein pathology would be very attractive in LBD. Commercial assays for measurement of α-synuclein have been available for some years. The total α-synuclein concentration is decreased by approximately 10 – 15% in CSF in early, untreated PD patients compared to controls, it is also decreased in DLB and PD compared to AD, however with significant overlap between patient groups[7, 133, 134]. It has been suggested that specific α-synuclein species, such as oligomers or phosphorylated α-synuclein might have a closer relationship to the pathology and prove to be superior biomarkers[7]. Differences in oligomeric α-synuclein in PDD compared to PD have been suggested[135]. However, these are still new concepts and more research is needed before any conclusions can be drawn.

Imaging- and nuclear medicine biomarkers

The dopaminergic neurons in substantia nigra pars compacta that degenerate in LBD project to the striatum[81]. The loss of dopaminergic transmission to the striatum can be assessed with dopaminergic imaging, most commonly using ligands that bind to presynaptic dopamine transporters[81]. The amount of binding is assessed with SPECT or PET, DAT is now a well-established PD and DLB biomarker[62, 81]. In addition to LBD, DAT imaging can be abnormal in MSA, progressive supranuclear palsy (PSP), and FTD as DAT is not a synucleinopathy marker, and will therefore be abnormal if there is another pathology in the striatum[62, 136]. Against AD, ^{123}I-FP-CIT DAT imaging (commercially DaTSCAN) had a sensitivity of 80% and specificity of 92% for DLB in autopsy-verified cases, thus performing better than clinical diagnosis in the same study[136]. It is important to point out that a normal DAT imaging does not rule out DLB. Normal DAT imaging could be explained the location of pathology at presentation, some patients likely have predominantly limbic and neocortical affection with little striatal neuronal loss[136]. In addition, a diffuse loss of dopamine across the striatum instead of a more specific loss in putamen may result in a normal scan[62].

Figure 2: DAT imaging (^{123}iodine-FP-CIT-SPECT) in AD, DLB and normal control (NC)



Reduced uptake in the caudate and putamen in DLB compared to AD and normal control. Reproduced with permission from McKeith et al[62].

18F-FDG PET traces glucose metabolism in different regions of the brain. Both AD and DLB patients have reduced uptake in temporal and parietal lobes[137]. Compared to individuals with AD, DLB patients generally have a more extensive occipital loss, while AD patients have reduced uptake in the medial temporal lobe[137]. In clinically diagnosed patients, visual ratings of FDG PET has been shown to have a sensitivity

and specificity of 74% and 70% respectively for separating AD from DLB patients[137]. The "cingulate island sign", that is relative preservation of metabolism in mid-or posterior cingulate gurus, has been shown to differentiate DLB from AD[62, 137]. DLB and PDD patients have similar patterns of glucose metabolic changes[112]. Amyloid imaging (see section 1.3.5) show that more DLB than PDD patients have equally severe beta-amyloid deposits as AD patients (assessed with PiB PET)[138]. In DLB deposits are seen in > 50% of cases, limiting its usefulness as a biomarker separating DLB and AD[138]. Higher PIB-retention were found to correlate with progression from PD with normal cognition to PD with mild cognitive impairment (PD-MCI), and conversion from PD-MCI to PDD[139].

The autonomic deinnervation in DLB can be visualized using ^{123}Iodine-MIBG myocardial scintigraphy. Comorbid conditions and medication use must be taken into consideration while interpreting results[62]. Polysomnographic verification of RBD in a patient with dementia is highly indicative of α-synuclein pathology, which is useful against AD but cannot differentiate between PD, MSA, and DLB[140]. Medial temporal lobe atrophy is another feature that can be used in differentiating between AD and DLB, as the medial temporal lobe is relatively preserved in DLB[62]. A visual rating scale of the medial temporal lobe on MRI had a sensitivity of 64% and specificity of 68% for DLB vs AD in autopsy verified cases[141]. However, combinations of AD and Lewy body pathology are common and medial temporal lobe atrophy cannot rule out Lewy body pathology[62].

Structural MRI findings associated with cognitive impairment in PD include hippocampal atrophy, and cortical volume loss in posterior, parietal and frontal regions[112]. Patterns of cortical thinning can predict conversion from PD to PD-MCI, and with the development of PDD the cortical thinning is more severe[112]. Specific EEG patterns have also been suggested as LBD biomarkers. Studies have reported an association with cognitive impairment in PD[135], ability to predict progression to DLB in MCI[135], and differentiate DLB from AD[62], as well as a correlation with clinically observed cognitive fluctuations[62].

1.5 Diagnosing dementia

1.5.1 Clinical and biomarker diagnosis

In addition to fulfilling the criteria for the dementia syndrome (see section 1.1), the relevant differential diagnosis must be considered and the specific dementia disease must be ascertained. Information from a carer/person with specific knowledge of the patient is necessary for assessment of the patient's level of functioning and the presence of neuropsychiatric symptoms, changes in emotional control, social behavior, and motivation, as the patient is often not a reliable assessor of his/her own function due to reduced insight and awareness as a consequence of the disease itself.

Cognitive testing must be broad enough to cover relevant domains, as well as standardized. Sensory impairment such as impaired vision or hearing must be corrected in the best possible manner, striving to make test results as representative as possible for the patient's current level of cognition. Importantly, all conditions that impair cognition could mimic dementia and thus careful history taking, including comorbid conditions and current medication is necessary. A general clinical and neurological examination should be performed. One important differential diagnosis that must be excluded is depression, which is common and can affect both cognitive function and activities of daily living. Structural intracranial pathology such as subdural hematoma, brain tumors, and normal pressure hydrocephalus must also be considered, and preferable MRI or alternatively a CT scan should thus be included. Structural imaging is also necessary to diagnose vascular dementia. Routine blood tests are also performed to exclude other conditions that could mimic dementia. Dementia differential diagnosis has traditionally been based on solely clinical criteria. However, newer research criteria include biomarkers[33, 57, 62], and biomarkers such as the volume of the hippocampal region and CSF AD markers t-tau, p-tau181 and abeta42, DAT imaging, and FDG and amyloid PET are routinely included in clinical workups[1]. The use of CSF biomarkers varies considerably globally, in the Netherlands and Sweden 40% of newly diagnosed dementia patients had a lumbar puncture[1]. The American Academy of Neurology advises that CSF markers are included for dementia

patients < 65 years, and the European Federation of Neurological Societies recommends that markers analysis in patients with an atypical AD presentation[1]. Both CSF markers and amyloid PET are also used to differentiate prodromal AD from the heterogenous MCI group[1].

1.5.2 Neuropathological dementia diagnosis

Neuropathological dementia diagnosis requires macro- but particularly microscopic assessment of brain tissue. All the pathologies must be recorded and in the end, a conclusion is drawn about which pathology is responsible for the dementia syndrome. The emergence of immunohistochemistry has been a major advantage for the detection of the different types of pathology present in dementia. As an aid in this process and to secure comparability between centres, standardized protocols have been developed for brain dissection, macroscopic description, which regions to analyse, and for tissue processing and staining[14, 142-145].

The neurofibrillary tangles stages from I-VI introduced by Braak and Braak in 1991[18] and the neuritic plaque staging by the CERAD[19] (see Section 1.3.2) have been central for neuropathological AD diagnosis. The National Institute on Aging and the Ronald and Nancy Reagan Institute of the Alzheimer`s Association neuropathological AD criteria from 1997 combines the Braak and CERAD stagings, while also acknowledging that patients might have more than one type of pathology with impact on the clinical presentation. The recommendations include a *grading of likelihoods that dementia in a patient is caused by AD*, from *high* in patients with both neuritic plaques and neurofibrillary tangles in neocortex (Braak V/VI and frequent neuritic plaque score according to CERAD), to *intermediate* in patients with moderate neocortical neuritic plaques and neurofibrillary tangles in limbic regions (Braak III/IV and CERAD moderate) and *low* in patients with a limited distribution and severity of neuritic plaques and neurofibrillary tangles (Braak I – II, CERAD infrequent)[25].

These criteria were again revised in 2012[14]. On a conceptual level the new criteria emphasised the difference between neuropathological AD lesions, regardless of clinical setting, and "Alzheimer´s disease", the clinical syndrome associated with significant AD neuropathology with a pre-clinical, a mild cognitive impairment and a dementia phase[14]. The revision also stressed the need to evaluate and recognize other kinds of pathology that might contribute to dementia. On a more practical level, the 2012 criteria include also an Aβ-plaque score modified from Thal et al[146] in addition to the neurofibrillary tangle score and neuritic plaque score based on Braak[18] and CERAD[19], and detailed recommendations for immunohistochemistry.

As mentioned previously (in section 1.4.3), both the 1996[60], 2005[61] and 2017[62] DLB criteria have recommendations for the pathological diagnosis of DLB. The 2005 and 2017 criteria also address the differential diagnosis versus Alzheimer´s disease, grading the likelihoods that the pathology found in a patient would be associated with the DLB clinical syndrome with varying degrees of AD pathology from low – intermediate and high. Whilst there are no generally accepted standard neuropathological consensus criteria for PD, traditionally an autopsy diagnosis has required degeneration of the substantia nigra pars compacta and Lewy body pathology[81].

In addition to ratings of amyloid, tau and α-synuclein aggregates, the presence of other pathology that might explain cognitive impairment such as TDP-43 proteinopathy often associated with FTD[147] and vascular lesions[148-150] should be carefully recorded before a neuropathological diagnosis is made.

1.6 Literature search

For this thesis, the comprehensive literature searches ended on July 1st 2018. Some additional references were added while reviewing the text prior to submission in May 2019.

2. Aim and objectives

The general aim of this study was to increase our understanding of pathological mechanisms underlying important clinical features in neurodegenerative cognitive impairment, by exploring the associations between clinical presentation, biomarkers, and neuropathology.

Objectives:

1) To explore whether there is a link between CSF biomarkers and neuropsychiatric symptoms in early AD.
2) To examine whether early cognitive impairment in PD was associated with α-synuclein, abeta42, t-tau or p-tau181 in CSF.
3) To examine whether clinical DLB criteria can predict DLB-pathology at autopsy.

3. Materials and methods

3.1 Subjects and samples

Table 3: Subjects and samples paper I, II and III

	Paper I	Paper II				Paper III	
Cohort	DemWest	Parkinson's Progression Markers Initiative (PPMI)				DemWest	
	AD n=32	PD n=414	Healthy controls n=189	PD-MCI n=140	PD normal cognition n=274	Autopsy group n=56	Non-autopsy n=149
Age in years	73.9 (9.0)	61.3, (9.7)	60.2 (11.3)	63.1 (9.3)	60.3 (9.7)	74.0 (8.2)	77.2 (6.8)
Sex (% male)	29% (9/32)	66% (273/414)	63% (120/189)	67% (94/140)	65% (179/274)	48% (27/56)	45% (67/149)
MMSE#	24.2 (2.3)					24.2 (2.7)	23.5 (2.7)
MoCA#		27.2 (2.3)	28.2 (1.1)	25.4 (2.5)	28.1 (1.6)		

Data are mean and standard deviations. #Results from the baseline. Abbreviations: PD-MCI: PD patients classified as MCI. AD=Alzheimers disease, PD=Parkinson's disease, MMSE= Mini-Mental State Examination, MoCA= Montreal Cognitive Assessment

3.1.1 The Dementia study of Western Norway (Demvest)

All patients referred to specialist clinics in Old Age Psychiatry and Geriatric Medicine in Rogaland and Hordaland counties in Western Norway between 2005 and 2007 for dementia workups were screened, and those diagnosed with mild dementia were asked to participate. All Neurology clinics in the region were also invited to refer patients to the study. From 2007 - 2013 we selectively recruited DLB and PDD patients. Inclusion criteria were mild dementia, defined as a Mini Mental State Examination (MMSE)[151] $\geq$ 20 and/or Clinical Dementia Rating Scale[152] of ≤ 1. Exclusion criteria were delirium, previous bipolar or psychotic disorder, terminal illness or recent diagnosis of a somatic disorder that could affect cognition, function or study participation.

The AD patients among the first 50 patients who underwent lumbar puncture were included in paper 1, n=32. 256 patients were originally included in the Demvest study, 18 patients withdrew, two proved not to have dementia and three never came to the

baseline evaluation. The neuropathology paper was based on the 56 patients who had come to autopsy by November 14. 2016, the autopsy cohort was compared to the patients who had died and not come to autopsy (n=149). At that time, 37 patients were still alive. One patient from the autopsy group was later excluded from the study as his cognitive impairment had not progressed after diagnosis.

3.1.2 Parkinson's Disease Progression Markers Initiative (PPMI)

PPMI is a clinical cohort study of newly diagnosed PD patients > 30 years old from 24 clinical sites in Europe, Israel and the United States. The study protocol and Case Report Form are published at ppmi-info.org, and data are publicly available through application to the Data and Publications Committee of the PPMI. Inclusion criteria were either asymmetric resting tremor or asymmetric bradykinesia, or two of the following; resting tremor, bradykinesia and rigidity, a PD diagnosis of two years or less at inclusion, a Hoehn and Yahr stage[153] of I or II at baseline and positive DAT imaging[154]. Use of PD medication was an exclusion criterion, and for inclusion the patients should not be expected to require medication within the first 6 months[154]. The PPMI also included a healthy control group. Inclusion criteria for controls included no first-degree family member with PD, no significant neurological disorder and a Montreal Cognitive Assessment (MoCA) > 26[155]. Dementia was an exclusion criterion for the PD group. For both groups any conditions or drug that could interfere with dopamine transporter imaging such as neuroleptics and metoclopramide, or the safety of lumbar puncture, such as the use of anticoagulants were reasons for exclusion.

In total there were 423 patients and 196 cognitively normal subjects in the PPMI database at the time of download in June 2014. For 6 patients and 7 controls, no CSF results were available from either the baseline or the first study visit three months later, and these were thus excluded. Three PD patients were excluded due to incomplete test results, resulting in 414 PD patients and 189 control subjects available for analysis.

3.2 Patient assessment

3.2.1 Demvest

The baseline evaluation was done by a study clinician (Geriatrician, Old Age Psychiatrist or Neurologist) with help from a research nurse, and consisted in addition to a structured interview of patients and caregivers of a neuropsychological test battery, neuropsychiatric evaluation, standardized assessments of DLB symptoms, a general physical and a neurological examination, ECG, routine blood tests and assessment of dementia severity with the Clinical Dementia Rating Scale[152]. Caregivers completed the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE)[156]. Activities of daily living were assessed with the Rapid Disability Rating Scale-2[157].

The MMSE[151] was used as a screening test of cognitive function. Verbal memory was tested using the California Verbal Learning Test[158], language was tested using the Boston Naming Test[159], visuospatial function by the Cube and Silhouette subtest of the Visual Object and Space Perception Battery[160] and the clock-drawing test, executive function by categorical verbal fluency (asking the patient to name as many animals as possible in one minute), the Trail Making Test A and B[161] and the Stroop Colour-Word test[162]. The neuropsychiatric evaluation consisted of the Montgomery and Aasberg Depression Rating Scale[163], where 10 items are scored from 0 (not present) to 6 (maximum severity), the Apathy Scale[164] where 14 items are scored from 0 (not present) to 3 (maximum severity), and the Neuropsychiatric Inventory (NPI)[165]. The NPI is a standardized interview of an informant, usually a spouse or off-spring with detailed and long-term information about the patients, about neuropsychiatric symptoms. The informant was asked about the presence of neuropsychiatric symptoms during the last four weeks. If the answer on a screening question was positive, the carer was asked to rate frequency from 1 - 4 (<1 a week, approximately once a week, more than once a week or every day) and intensity of these symptoms, based on how much these symptoms negatively affect the patient from 1- 3 (mildly, moderately or severely). Frequency and intensity are then multiplied, resulting in a 1-12 score, where

12 is maximum severity for each of the items, there are 12 items in total. Psychosis was defined in this study as the combination of the three items delusions, hallucinations, and agitation.

Parkinsonism was rated by the United Parkinson's Disease Rating Scale (UPDRS) motor subscale[166] – a diagnosis of parkinsonism required two of the following bradykinesia, tremor, rigidity, and gait disturbance/postural instability. Fluctuations were assessed by the Clinician Assessment of Cognitive Fluctuation[108] and the Mayo Fluctuation Questionnaire[107], applying recommended cut - off scores. RBD was evaluated using the Mayo Sleep Questionnaire[117], in which the bed partner answers specific questions about dream enactment behaviour; duration of symptoms, injuries to patient or bed partner, dream content and if movements match dream content. The structured interview included a question about any adverse reactions to antipsychotics.

A structural MRI was performed in cases without contraindications; in those instances, a CT scan was conducted instead. According to the study protocol, all participants where the clinician suspected that DLB could be a differential diagnosis should have been referred to DAT imaging, but due to availability this was not possible in all cases.

After the baseline evaluation, participants were followed up with an annual examination until death. These evaluations were similar, but less extensive, to that preformed at baseline. Biannual meetings were held between study clinicians to ensure inter- and intra-rater reliability and harmonize procedures for the first 10 years of the study.

3.2.2 PPMI

Data from the screening visit and the baseline visit (completed within 45 days after the Screening visit) in PPMI were used in this study, in addition to CSF results from either baseline or the visit 3 months after baseline. For both patients and controls, these first two visits included a structured interview, neuropsychological and neuropsychiatric evaluations, general physical and neurological examinations and routine blood tests.

PD patients also completed The Movement Disorder Society (MDS) Unified Parkinson´s Disease Rating Scale parts (UPDRS) I-III[167], disease staging with Hoehn and Yahr which was included in the MDS-UPDRS, and the Modified Schwab & England Scale rating ability to perform activities of daily living[168]. RBD, daytime sleepiness and other non-motor features of PD were also assessed.

Cognitive functions were assessed by the MoCA[155] and tests of different cognitive functions. Memory was tested using the Hopkins Verbal Learning Test Revised (HVLT)[169]. Visuospatial functions were assessed using the Benton Judgment of Line Orientation (the 15 item-version)[170], executive function by three semantic fluency tests (number of fruits, vegetables, and animals said in 60 seconds), attention by the Letter-Number Sequencing Test[171] and the Symbol digit modalities test[172]. For cognitive tests with continuous scores, the PD results were converted to z-scores using the means and standard deviations from the control group. Cognitive domains were created by averaging z-scores from different tests of the same domains. Subtests from MoCA[155] were combined with the other cognitive tests when creating domains, to include as much information as possible. Memory was constructed with the average of the z-scores for total immediate recall (encoding) and delayed recall (retrieval) on the HVLT[169]. Attention and executive function were combined in one domain (z-scores of Phonemic fluency (from MoCA[155]), Semantic Fluency, Symbol Digit Modalities Test[172], and Letter-Number Sequencing[171]). The visuospatial domain was constructed using the z-score of Benton Judgement of Line Orientation[173]. In addition to the three domains, we constructed a global composite cognition factor; a single regression-based factor score based on principal component analysis to reflect the common variance on all available cognitive tests in the whole sample of controls and patients. This composite cognition factor represented 34% of the variance in the whole test battery. With regards to imaging, structural MRI brain scan was available in addition to the DAT imaging.

3.3 Clinical diagnostic procedures

3.3.1 Demvest

Dementia was diagnosed according to the DSM IV[174] criteria, which are similar (but not identical) to the ICD-10[4] criteria previously listed. The different dementia diseases were diagnosed according to the relevant research criteria available at study start in 2005. Alzheimer's disease was diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria[175], DLB according to the 2005 DLB Consortium criteria[61], vascular dementia the National Institute of Neurological Diseases and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria[176] and dementia in Parkinson's disease according to the recommendations from the Movement Disorders task force[63]. Alcohol dementia was diagnosed using the criteria from the DSM IV[174], and frontotemporal dementia according to the Lund-Manchester criteria[177]. The first clinical diagnosis was made after the baseline visit, two psychiatrists with long experience in dementia diagnosis independently applied the diagnostic criteria based on all the information from the baseline visit including the case note from the clinician. In the event of disagreement, cases were discussed until consensus was reached. The clinical diagnosis were reviewed after both two and five years. The latter consensus process also involved an experienced geriatrician. The revisions focused on participants with previous "possible" AD/DLB/VaD diagnoses, or an unexpected clinical course, for instance, slow cognitive decline. All available information, including clinical records and neuroimaging, but excluding neuropathology were used in these revisions.

3.3.2 PPMI

A clinical diagnosis of PD within the last two years was required. The case report form has a specified list of diagnostic features increasing and decreasing the likelihood of PD and the clinician was required to note the presence or absence of each feature. Based in this, the clinician concluded as to whether the patient was likely to have PD. Positive DAT imaging was a requirement for inclusion as a PD subject.

PPMI was designed prior to the publication of the MDS task force criteria for MCI in PD[178], and the neuropsychological test battery does not include two independent tests of each of the five cognitive domains as required for Level II "Comprehensive Assessment" in these criteria. Therefore, an approximation to these criteria was used, where subtests of MoCA[155] were included as described previously[179] to create the required five domains; executive function, attention and working memory, memory, visuospatial function, and language[178].

The memory domain was assessed by the total and immediate-delayed recall from HVLT[169], the executive function domain using the semantic fluency tests in addition to phonemic fluency and the Trail Making item from MoCA, language by abstraction, sentence repetition and naming from MoCA, visuospatial functions using the clock and cube from MoCA in addition to the Benton Judgment of Line Orientation[173], and finally attention was based on backward digit span, vigilance and serial 7s from MoCA in addition to the Symbol Digit Modalities Test[172] and the Letter-Number Sequencing[171]. Impairment was defined as a result more than two standard deviations below the results in the control group, or inability to complete the MoCA item. Patients were defined as having MCI if they showed impairment on two or more tests, single domain MCI if both tests were within the same domain or multiple domain if there were impairment on tests across domains[178]. The PD patients with impairment on ≤1 test were classified as PD with normal cognition.

3.4 Biomarker assessment - CSF

3.4.1 Demvest

Lumbar puncture was performed between 07 00 – 10 00, with the patients having fasted from midnight. The samples were kept on ice and transported to the laboratory where the first 3 mL were used for routine analysis of glucose, protein and cell count. Study samples were collected directly into polypropylene tubes, centrifuged at 2000 *g* for 10 minutes and the supernatant transferred to new polypropylene tubes. Samples

were frozen at -80 and stored until shipment on dry ice to the central storage. Samples went through one freeze-thaw cycle for aliquoting to 0.5 mL tubes prior to analysis. Only polypropylene tubes and pipette tips were used.

Total tau and tau phosphorylated at threonine 181 were analyzed using the commercially available Innotest enzyme linked immunosorbent assays (ELISAs) by Innogenetics (originally from Ghent, Belgium – merged with Fujirebio, Japan in 2010). The abeta42 ELISA came from Biosource Europe S.A. Analysis were done according to the manufacturer`s protocols at a research laboratory at Stavanger University Hospital.

These assays are sandwich ELISAs, the principles of which are explained briefly here; a capture antibody is bound to the solid phase – the bottom of the wells of a plastic plate. The sample is added and the peptide/protein binds to the capture antibody, after this stage the plate is washed to remove any peptide/protein not bound to the capture antibody. A secondary antibody against the peptide/protein is added, this antibody is labeled with the substrate for an enzyme. The plate is washed again to remove any secondary antibody not bound to a peptide/protein. An enzyme is added, and the interaction with the substrate produces a color change that is detected by a reader that measures absorbance at a specified wavelength of light, ”color intensity”. Standards with known concentrations of the protein /peptide in question are used to create a curve with absorbance vs concentration, and from this curve the concentration in the sample is calculated based on the absorbance.

For t-tau, p-tau181 and abeta42 the standard range was 75 – 1200 pg/mL, 15.6 – 500 pg/mL, and 15.6 – 1000 pg/mL, limits of detection were 59.3 pg/mL, 15.9 pg/mL and < 10 pg/mL respectively. Intraassay coefficient of variation (%CV) were 1.2 – 5.9 in the t-tau assay, < 5.0 in the p-tau181 assay and ~3 in the abeta42 assay, inter-assay CV were 1.7 - 6.0%, <10% and <5.5% CV respectively.

3.4.2 PPMI

CSF was collected in siliconized polypropylene tubes and the first 1-2 mL sent to the local laboratory for routine analysis of cell count, total protein levels and glucose level. The next 15 – 20 ml was centrifuged at 2000 *g* for 10 minutes at room temperature, and then transferred into precooled 1.5 ml tubes that were immediately frozen using dry ice. The frozen aliquots were shipped overnight to the PPMI Biorepository Core laboratories on dry ice. Samples were thawed there and aliquoted into 0.5 ml siliconized polypropylene tubes and stored at -80° C until analysis.

Abeta42, t-tau and p-tau181 were analysed as described previously[180, 181] using the multiplex Luminex xMAP platform (Luminex Corp) with INNOBIA AlzBio3 (Fujirebio-Innogenetics, Ghent, Belgium) kit reagents at the University of Pennsylvania. The Luminex assays are based on the capture antibody being bound to beads instead of the bottom of the well as a traditional sandwich ELISA; one type of bead is covered with a specific type of target antibody, and several kinds of beads can be added to the same sample, thus having the major advantage of allowing for the concomitant analysis of several peptides/proteins. In the last step, one laser frequency recognizes the type of bead and a second laser the amount of bound protein to that specific kind of bead (for more information, www.luminexcorp.com). Briefly, 75 µl of samples, standards, aqueous controls and two pooled CSF samples used for quality control were analysed in duplicate. After 38 runs, mean variability of concentrations (%CV) were 9.0, 6.7 and 8.2 for abeta42, t-tau, and p-tau181 respectively in the pooled CSF samples. The results for t-tau in 6 baseline samples (four PD and two healthy controls) did not meet quality requirements and were excluded from further analysis. A-synuclein and CSF haemoglobin levels were analysed using commercially available sandwich ELISA kits from Covance (Dedham, MA) at Covance. A total of 81 runs were conducted, and the CV for two quality control CSF samples was 17% for α-synuclein. The haemoglobin assay had standards in a range of 7.5 – 125 ng/ml, and samples were analysed at 3 dilutions to secure that at least one dilution fell within the assay range.

As the levels of α-synuclein in blood cells are much higher than in CSF, blood contamination of CSF, which is not uncommon, may influence results[182]. 306 controls and PD patients had CSF haemoglobin values below the detection limit, and for 131 no CSF haemoglobin values were available at the date of data download. For some individuals, more than one CSF haemoglobin value was available, in these cases, the one closest to the date of the α-synuclein analysis was selected.

As expected, CSF α-synuclein and CSF haemoglobin concentrations were correlated (all PPMI samples, including subjects without evidence of dopaminergic deficits, Spearman Correlation coefficient 0.092, p=0.036). A more detailed analysis of the data suggested correlation was driven by five outliers with extreme α-synuclein values (> 3 standard deviations (SD) above the mean). After exclusion of these outliers, there was no longer a significant correlation between α-synuclein and haemoglobin. To further explore the relationship between α-synuclein and haemoglobin, the dataset was subdivided into 4 groups; haemoglobin below the detection limit, haemoglobin > detection limit < 200 ng/ml, haemoglobin > 200 ng/ml and haemoglobin missing. A Kruskal-Wallis test for independence was performed, and CSF α-synuclein values were found to be similar in these groups. Thus the five PD patients with α-synuclein > 3 SD above the mean were excluded from further analysis.

DNA was extracted from whole blood, and *APOE* genotypes determined using allele-specific oligonucleotide probes (TaqMan method). TaqMan assays were used according to the manufacturer's instructions (Applied Biosystems, Foster City, CA)[180].

3.5 Neuropathological diagnostic procedures

3.5.1 Demvest

The details of the sampling of brain regions, immunohistochemistry and antibodies are described in the original paper. Briefly, the BrainNet Europe and Brains for Dementia Research UK protocols were followed for brain dissection, choice of brain regions,

tissue processing, and staining and block taking for both histological, immunohistological and neuropathological assessments[14, 142-145].

Specific stains were used for immunohistochemical characterization of β-amyloid (diffuse and classical plaques and amyloid angiopathy), hyperphosphorylated tau (dystrophic neurites, pretangles, tangles, and neuropil threads) and α-synuclein pathology (Lewy neurites and Lewy bodies) according to the protocols mentioned above. Any TDP-43 pathology was assessed according to the appropriate guidelines[147], and vascular lesions such as small vessel disease and infarctions were recorded[148].

Cases were evaluated by an experienced neuropathologist (TH) who was blinded for all clinical information. Neuropathological diagnoses were made according to accepted consensus criteria for AD[14, 18, 19, 25], DLB[61, 82, 144] and VaD[149, 150]. Patients were classified as having LBD if the likelihood of the pathology being associated with the DLB syndrome was intermediate or high according to the 2005 McKeith criteria[61]. Cases diagnosed neuropathologically as LBD were given a pathological diagnosis of DLB if the clinical diagnosis was DLB and PDD if the clinical diagnosis was PDD. One case was difficult to classify according to the protocols mentioned previously, with Lewy body pathology displaying an atypical pattern of distribution. The Lewy body pathology was labeled “mostly limbic”, and the case diagnosed as AD due to severe AD with Braak neurofibrillary tangle stage VI.

3.6 Statistical analyses

3.6.1 Simple comparisons and correlation analyses

Data were analysed using several editions of SPSS Statistic. $p < 0.05$ was considered statistically significant. When demographic variables and CSF marker results were compared between groups the Student´s t-test, the Mann-Whitney test, Fischer`s exact test or Chi-square test were used as applicable. The distribution of the original data was checked using the Kolmogorov-Smirnov statistics and simple scatter plots. When data were not normally distributed, such as CSF marker results in both the Demvest

and PPMI cohorts non-parametric analyses were used including Spearman correlations. Sensitivity and specificity were calculated using appropriate formulas and 2 x 2 tables, see table 4[183]. Cohen´s *kappa* (κ) measure of agreement was calculated as follows, using the letters from table 4 in the equations. Observed measure of agreement = $Io = (a+d)/n$. Expected measure of agreement = $I_e = ((a+c)(a+b)+(b+d)(c+d))/n^2$. Cohens $\kappa = (I_o-I_e)/(1-I_e)$[183].

Table 4: 2x2 table with formulae[183]

		Test (clinical diagnosis)		
		Positive	Negative	
True (pathological diagnosis)	Positive	a Number of true positives	b Number of false negatives	Sensitivity=a/(a+b)
	Negative	c Number of false positives	d Number of true negatives	Specificity=d/(c+d)
		Positive predictive value=a/(a+c)	Negative predictive value=d/(b+d)	n=a+b+c+d

3.6.2 Regression analysis

As mentioned in 3.2.2, cognitive domains (attention/executive, memory, visuospatial functions) were created by combining tests of the same domain, in addition to a composite cognition factor representing common variance on cognitive tests. To explore whether the CSF markers influenced cognition, four different multiple linear regression models were constructed, with the cognitive domains and the composite cognition factor as dependent variables. As a first step, only the CSF markers were added. As a next step, the models were adjusted for potential confounders including age, gender, education and *APOE* status (presence of an *APOE* ε4 or ε2 allele), in addition to total MDS-UPDRS III score[167]. *APOE* genotype did not prove significant in any of the models and was thus not included in the final analysis (see table 6 for the final models). To compare CSF marker concentrations between PD-MCI and PD with normal cognition, multinomial logistic regression was conducted. The analysis was

adjusted for other factors with possible impact on cognition, such as gender, age, education, the severity of PD rated with the MDS-UPDRS III[167] score and other markers. The predictors were added in different fashions, demographics and MDS-UPDRS III[167] were added as forced entry, while the other CSF markers were added stepwise. The regression analyses were checked for multicollinearity using as recommended by Belsley and Kuh[184], and also for leverage, heteroscedasticity and normality of residuals, and did not violate assumptions. However, there was a trend towards non-normality of the residuals in the analysis of the visuospatial variable.

3.6.3 Missing data

All the 32 AD patients in paper I had results on all three CSF markers. The Spearman correlations were explored in all patients with available neuropsychiatric assessment results, without any adjustments for missing data. In the PPMI cohort, only patients without missing values were included in the analysis (n=403), (5 were excluded because of extreme α-synuclein values, two due to missing p-tau181, and 4 due to missing t-tau results). 38 PD patients had no *APOE* results, but as mentioned *APOE* genotype was not included in the final models. Thus the patients included in the models had complete data sets for both the demographic variables, neuropsychological tests, and CSF marker results.

Four of the autopsied (n=56) and six of the non-autopsied patients (n=149) in the Demvest cohort were missing information about disease duration prior to diagnosis. Demographical information, baseline MMSE[151] scores and disease duration from diagnosis to death the data were available for all.

3.7 Ethical approval

The project followed the recommendations from the Helsinki declaration. The Demvest study was approved by the regional committee for medical and health

research ethics in western Norway (REK 2010/633). The patients all had mild dementia at inclusion and were thus considered able to consent on their own behalf. The study procedure was explained to all patients in the presence of a carer, and the study participant provided written consent. All patients were asked to consent for both lumbar puncture and autopsy, however it was also possible to participate in only the clinical part of the study. The PPMI study was approved by the medical ethical committees at the respective centres. Written informed consent was obtained from all participants, and as dementia was an exclusion criterion all were considered able to consent.

4. Results

4.1 Results paper I

Our sample were majority female (71% women), mean age was 73.9 years (SD 9.0). Mean duration of disease since the first symptoms were noted by carer or patient were 2.1 years (SD 1.1), and the patients had mild dementia with a mean MMSE score of 24.2 (SD 2.3). We found significant correlations between the t-tau and p-tau181 concentrations and apathy, assessed with Starkstein apathy scale[164], see table 5 and figure 3.

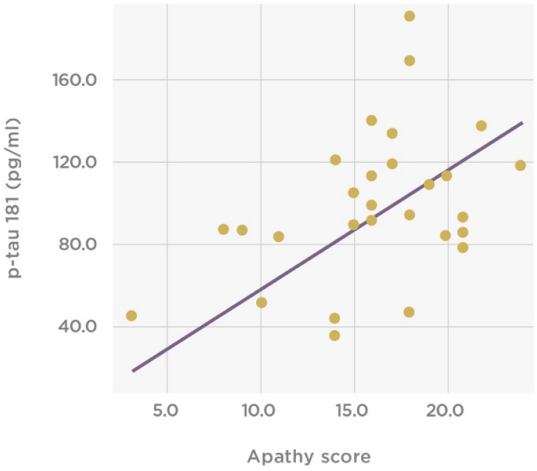
Table 5: Correlations between CSF biomarkers and neuropsychiatric outcome measures, Spearman's rank correlation coefficients

	Depression	Apathy	Psychosis (NPI-3)
Total tau	0.16	0.38*	-0.004
p-tau181	0.01	0.38*	-0.05
Abeta42	-0.07	0.13	0.08

Abbreviation: NPI=Neuropsychiatric Inventory. NPI-3: delusions, agitation and hallucinations items. *p<0.05

CSF abeta42 was not associated with any of the neuropsychiatric symptoms. No significant correlations were found between depression and psychosis and the markers, neither when the NPI[165] delusions, agitation, and hallucination items were analysed together or when the items were analysed separately.

Figure 3: Correlation between apathy scores and CSF p-tau 181 levels in AD



4.2 Results paper II

CSF biomarker results and MCI classification

CSF α-synuclein, t-tau and p-tau181 concentrations in CSF were significantly lower in the 414 PD patients compared to the 189 healthy controls ($p<0.001$, $p=0.001$ and $p=0.001$), in contrast abeta42 concentrations were similar. T-tau and α-synuclein correlated significantly with age in the whole cohort of patients and controls (Spearman´s correlation coefficient 0.209, $p<0.001$ and 0.123, $p=0.002$ respectively), and in the PD patients (Spearman´s correlation coefficient 0.276, $p<0.001$ and 0.140, $p=0.004$), while in the control group only t-tau correlated with age (Spearman´s correlation coefficient 0.337, $p<0.001$).

140 PD patients (34%) had MCI and the remainder normal cognition. Of those with MCI, 7% had single domain and 93% had multiple domain MCI. The PD-MCI patients were older ($p=0.005$), less educated ($p<0.001$) and had more severe motor impairment reflected in higher UPDRS-III[167] scores ($p=0.013$) than the other PD patients. Abeta42 was significantly reduced in the PD-MCI group compared to healthy

controls (p=0.033) after adjusting for age, education, the severity of PD and the effect of the other CSF biomarkers, but no significant difference was found between PD patients without cognitive impairment and controls. The CSF concentrations of α-synuclein, t-tau and p-tau181 were significantly lower in both the PD-MCI and the PD patients with normal cognition compared to healthy controls, but no differences were found in marker concentrations between PD-MCI patients and PD patients with normal cognition.

Relationship between CSF markers and cognition

The next stage was to analyse the associations between the composite cognition factor and cognitive domains and the CSF markers in multiple regression analysis (Table 6). In the models adjusted for all covariates (age, education, gender, total UPDRS-III[167] score), low α-synuclein was significantly associated with reduced cognitive function both assessed with the composite cognition factor (standardized beta =0.132, p= 0.019) and the executive function / attention domain (standardized beta = 0.137, p=0.020). Reduced α-synuclein was also associated at trend level with both the memory (standardized beta 0.109, p= 0.068) and visuospatial domains (standardized beta=0.118, p=0.058).

Increased t-tau was significantly associated with decreased function in the executive/attention domain (standardized beta= -0.216, p=0.001) and visuospatial function (standardized beta=-0.181, p=0.008), but the associations were no longer significant when the other covariates were added to the model. No significant associations were found for abeta42 and p-tau181 and cognition.

Table 6: Results from the multiple regression analysis in the PD patients

		Composite cognition		Executive/ attention		Visuospatial function		Memory	
Step	Predictor	Beta	P	Beta	p	Beta	p	Beta	p
1.	abeta42	0.071	0.189	0.068	0.211	0.067	0.220	0.020	0.719
	t-tau	−0.187	**0.006**	−0.216	**0.001**	−0.181	**0.008**	−0.128	0.062
	p-tau181	0.038	0.487	0.050	0.364	−0.008	0.891	0.028	0.618
	α-synuclein	0.165	**0.014**	0.174	**0.009**	0.161	**0.016**	0.136	**0.044**
2.	abeta42	−0.015	0.752	−0.005	0.922	0.064	0.214	−0.055	0.259
	T-tau	−0.058	0.327	−0.084	0.172	−0.094	0.155	−0.023	0.715
	P-tau181	0.029	0.525	0.037	0.443	−0.026	0.612	0.022	0.657
	α-synuclein	0.132	**0.019**	0.137	**0.020**	0.118	0.058	0.109	0.068
	Gender (1 = F, 2 =M)	−0.251	**<0.001**	−0.153	**<0.001**	0.230	**<0.001**	−0.246	**<0.001**
	Age (in years)	−0.382	**<0.001**	−0.372	**<0.001**	−0.132	**0.009**	−0.323	**<0.001**
	Education (in years)	0.294	**<0.001**	0.294	**<0.001**	0.238	**<0.001**	0.256	**<0.001**
	UPDRS III total	−0.104	**0.014**	−0.062	0.158	−0.116	**0.014**	−0.085	**0.057**

Results from the multiple linear regression models of the effects of CSF markers on cognition in the PD patients. In step 1 only the CSF markers were included as predictors vs composite cognition and the cognitive domains as dependent variables. In step 2 potential confounding variables were added. The reported beta-values are standardized.

4.3 Results paper III

Demographics and correlation between clinical and pathological diagnosis

Gender, education and baseline MMSE were similar in the autopsy cohort (n=56) and the non-autopsy cohort (n=149) (see Table 1 in the original publication). The autopsy group was somewhat younger at baseline (mean age 74.0, SD 8.2 versus 77.2, SD 6.8, p=0.025) with longer survival after the baseline evaluation (mean 76.4 months, SD 29.0 versus 54.3 months, SD 30.6, p<0.001) than the non-autopsy cohort.

As it is not possible to separate DLB and PDD neuropathologically, these are referred to as LBD when reporting the neuropathological diagnoses. Of the 56 patients who came to autopsy, 31 received a neuropathological diagnosis of AD, 20 LBD, two

vascular dementia and one case each of progressive supranuclear palsy, TDP-43 positive frontotemporal dementia and no significant pathology. The patient without significant pathology had already been excluded from the main study because the cognitive impairment did not progress. One patient with Braak α-synuclein stage 3[82] and no other pathology (Braak tau stage I and only mild small vessel disease) was classified as LBD, with a clinical diagnosis of PDD. This patient was clinically a clear cut PD patient with response to levodopa, but increasing cognitive impairment and visual hallucinations. The patient died at 61, just one year after inclusion.

The neuropathologically confirmed LBD cases had a significantly shorter duration from diagnosis to death compared with the neuropathological AD patients, mean survival of 60.9 months (SD 27.8) and 87.0 months (SD 23.9, p=0.002) respectively. Age, education, baseline MMSE results, and disease duration prior to diagnosis were similar in the neuropathological AD and LBD groups.

Concomitant pathologies were common; 10 AD patients had some Lewy body pathology, including three restricted to the amygdala only. Varying degrees of AD pathology was present in nearly all LBD patients. TDP-43 pathology was common, found in 9 AD patients and 6 LBD patients. Vascular pathology, such as amyloid angiopathy and small vessel disease was common in both AD and LBD, some patients also had cerebral infarctions.

Among the 14 patients with clinical probable DLB, 11 had pathological LBD and three had AD. One patient had clinical possible DLB but pathological AD. Of the 20 patients with neuropathological LBD, 11 had a clinical DLB diagnosis, five a clinical PDD diagnosis, two had probable and two had possible AD. A clinical LBD diagnosis had sensitivity, specificity, positive and negative predictive values of 80%, 92%, 84% and 89% for DLB and PDD combined. If the PDD patients were excluded, clinical probable DLB had sensitivity, specificity, positive and negative predictive values of 73%, 93%, 79%, and 90% respectively. Diagnostic accuracy, i.e. the agreement

between clinical and neuropathological DLB diagnoses were 88%, Cohens κ measure of agreement was 0.67.

Table 7: Correlation between clinical and pathological diagnosis

		Clinical diagnosis								
		Prob AD	Prob DLB	Prob VaD	Mixed AD/VaD	Prob PDD	Poss AD	Poss DLB	Other	Total
Pathological diagnosis	AD	23	3	1	2	0	0	1	1 #	31
	LBD	2	11	0	0	5	2	0	0	20
	VaD	0	0	2	0	0	0	0	0	2
	Other	1 $	0	0	0	0	0	0	2 +	3
	Total	26	14	3	2	5	2	1	3	56

#The clinical diagnosis was alcohol related dementia.
+ One of these patients had clinical and pathological FTD, the other clinical MCI and a neuropathological diagnosis of aging and mild AD.
$ The pathological diagnosis was progressive supranuclear palsy.

Patients with "false negative" and "false positive" DLB diagnoses

Details about the neuropathology and the presence of core DLB features at the annual assessments in the "false negative" and "false positive" DLB patients can be found in Table 4 in paper III. None of the four false negative patients, that is patients with pathological but not clinical LBD had any of the core features at baseline. However, three of the four had no bed partner and polysomnography was not performed, thus the presence of RBD could not be properly assessed. UPDRS-III[166] scores were slightly elevated in two of the false negatives, 11 and 9 at baseline respectively, with significant progression at follow up. One of the four also developed cognitive fluctuations and visual hallucinations. MRI showed hippocampal atrophy in all of these cases. In the two patients without parkinsonism, one developed fluctuations and visual hallucinations from the third follow up which persisted. The other patient was entirely without core features until the 5-year follow-up, after which she developed both cognitive fluctuations and visual hallucinations. The pathological assessment showed severe AD pathology in all these false negative cases, with Braak tau stage VI[18] in addition to severe Lewy body pathology with neocortical Lewy bodies.

Three patients were false positives; meaning they were diagnosed clinically as LBD, but this was not verified at pathology. All three had both visual hallucinations and fluctuations at baseline, which persisted for several years. MRI showed relatively preserved hippocampal regions. Importantly, two of the patients had impaired vision, one of them on both eyes. Their parkinsonism increased gradually and was moderate to severe 3 years after baseline, but two of them had received treatment with antipsychotics. However, both these patients still fulfilled the DLB dignostic criteria also without the parkinsonism, due to the presence of fluctuations and visual hallucinations. Neuropathology showed that all three had severe AD with Braak tau stage VI. Whilst the two patients with eye disease had no Lewy body pathology, neurofibrillary tangle pathology was present in the substantia nigra, and in one of them TDP-43 pathology was present in the amygdala and hippocampus. These two patients were the ones with eye disease. The third patient had limbic α-synuclein pathology in addition to amyloid angiopathy and small vessel disease. This case did not readily fit into the McKeith DLB criteria[62], with Lewy body pathology skipping the substantia nigra, but with cortical involvement.

5. Discussion

This section will start with a discussion of our methods (5.1). I will review strengths and limitations in our patient sampling, clinical assessments, CSF analysis, neuropathological analysis and statistical methods and how this might have influenced results. The next segment (5.2) is a discussion of our results. I will compare our findings to those of others, discuss similarities and discrepancies, explore implications and conclude were this is prudent.

5.1 Methodological considerations

5.1.1 Samples and clinical assessments

Sampling of patients

The Demvest study is not population-based, but rather patients referred to specialist clinics in Geriatric Medicine and Old Age Psychiatry for dementia work-ups. An asset of the study is that all relevant specialist clinics in the Hordaland and Rogaland counties in Norway participated. However, in Norway, many persons with dementia are diagnosed by primary care physicians, and the patients referred to specialist clinics may not be representative for the total dementia population. An overrepresentation of patients with neuropsychiatric symptoms, other non-cognitive symptoms or an atypical course is likely. This might have influenced results in paper I.

The prevalence of DLB is higher in secondary than primary care settings[64]. For the first two years of the study period, all referrals were screened, resulting in 16% DLB of newly diagnosed dementia patients. This is higher than recent findings from specialist clinics in the UK were DLB constituted 5% of incident dementia cases[185], although in the population based Islington study DLB constituted 11% of dementia cases > 65 years[186]. Later DLB and PDD patients were recruited selectively in Demvest and are thus overrepresented. The Demvest cohort included 32% DLB and 7% PDD based on clinical diagnosis after 5 years. Prevalence does not affect estimates of sensitivity and specificity, but the high LBD prevalence increased our estimates of

the positive predictive value and decreased the negative predictive value in paper III compared to populations with lower LBD prevalence.

The PPMI patients were mainly from University clinics and thus not representative for all PD patients. They were younger than patients in other cohorts of newly diagnosed PD patients, with a mean age of 61.3 years compared to mean 65.9[187] and 67.6[67] years in two population-based cohort studies of newly diagnosed PD. Our PD and control groups were highly educated with a mean education of 15.6 and 16.0 years respectively. In comparison, 34% in the United States population born between 1948 – 1952 (corresponding to the age bracket of the majority of the PPMI patients) had completed a bachelor's degree or higher (corresponding to ≥ 16 years of education)[188]. Age and education are important factors in explaining why the PPMI patients had better cognition at baseline than other cohorts, mean MoCA score in PPMI was 27.1 compared to 25.3[187] and approximately 23-24 (converted from MMSE 27.8)[189, 190] in others. This might have decreased the strength of our associations, as higher education likely causes individuals to perform better cognitively even when harbouring pathology[1]. The requirement of a positive DAT imaging for inclusion as a PD patient separates our cohort from many other PD cohorts. This strengthens our ability to find significant associations if present, as it increases the probability that included patients have PD.

Clinical patient assessments

Strengths of both the Demvest and PPMI cohort include the extensive subject characterization and the long duration of follow-up in the Demvest. Few studies have followed patients from dementia diagnosis to death with yearly follow up as the Demvest study did. This gives an opportunity to follow the development of symptoms, unlike studies that are either based on diagnoses from a single baseline interview or have a long interval between the last follow-up and autopsy.

Limitations to the Demvest study include that only 50 of 242 were evaluated with DAT imaging, only 56 has yet come to autopsy. Presence of RBD was assessed using the Mayo Sleep Questionnaire[117], which is based on information from a carer. Thus

our ability to detect RBD depended on whether the carer had observed the patient sleep, and was likely lower in patients without a bed-partner. Polysomnography and DAT imaging in all patients would have increased the accuracy of clinical diagnosis.

When assessing neuropsychiatric symptoms in dementia some scales are rated by informants/carers and others by the patients themselves; these may measure different concepts. We applied a specific scale for apathy, whereas several other studies relied solely on the NPI[165] apathy item. A broader neuropsychological test battery in PPMI would have strengthened our cognitive domains. However, on the other hand, too many instruments and tests might increase the risk of missing data and drop-outs, and thus reduce the quality of the dataset. The multicenter design of both PPMI and Demvest with the involvement of a large number of clinicians increases the risk of both random and systematic errors of testing, application of standardized scales and the diagnostic processes. Random errors would decrease the strengths of our associations, thus making us prone to type II errors, systematic errors could make our results inaccurate. On the other hand, for paper III the multicenter design involving all relevant out-patient clinics in the catchment area makes results more applicable to general clinical practice than if the study were conducted in a tertiary centre specializing in DLB.

Subsamples vs the whole cohort in Demvest

Gender, baseline MMSE scores and education were similar in the autopsy and non-autopsy groups, but patients in the autopsy group were somewhat younger at inclusion and had longer survival. There are several possible reasons for this. Firstly, it took some time from study start until all logistics with the three participating pathological departments and undertakers had been properly established. Secondly, some patients required more time to reflect and discuss with family and did not consent to autopsy at the first visit. Thirdly, most of the patients with long survival lived in nursing homes where regular visits from study nurses and physicians and information about autopsy procedure in the patient files may have increased the likelihood of autopsy being carried out, compared to patients dying early from unrelated causes.

MCI classification in the PPMI

The PPMI design did not include a neuropsychological test battery sufficient for a level II MCI diagnosis according to the MDS MCI in PD criteria[178], thus we had to make adjustments which might have decreased the accuracy of the MCI classification. We report a MCI prevalence of 34%, in the newly diagnosed and untreated PD patients. Other studies using the level II MDS criteria have found a MCI prevalence of 20% - 65% in PD, but not all these patients were newly diagnosed[191]. Reports of MCI prevalence in newly diagnosed PD are somewhat conflicting. Some are similar to our findings (35%[192] and 33%[193]), while one population-based study a found higher prevalence (43%)[187] and another population-based study a lower prevalence (19%) in an older and less educated cohort[67]. Weintraub and coworkers found that 22% of the PD patients in the PPMI cohort fulfilled MDS level I criteria for MCI in PD, but only 9% were classified as MCI according to their approximation to the level II criteria[194]. The MDS level II MCI criteria define impairment as "performance between 1 to 2 standard deviations (SDs) below age, education culturally applied norms…"[178]. Weintraub and coworkers MCI classification was based on published norms for the neuropsychological tests, however, only two of the five tests were adjusted for education[194]. This may have led them to falsely classify highly educated PD patients with impaired cognition as cognitively intact. This, in addition to the fact that they chose 1.5 standard deviations below the meanwhile we chose 2 standard deviations, may partly explain the discrepant results.

The PPMI required controls to have a MoCA score above 26. A population-based Norwegian study of 63 – 65-year-olds (n=3413) found that mean MoCA score was 26.2 (SD 2.5) in the group with the highest level of education (> 12 years) and thus most comparable to the PPMI controls and patients[195]. Although test results improved significantly with education[195], and the PPMI patients and controls on average have more education than 12 years, there is a substantial risk that the PPMI control group might have *superior cognition* to even similarly educated populations. For PD patients, the criteria for cognition was merely no dementia, and the PD group had significantly lower total MoCA score than the control group[180].

Our MCI classification was based on comparisons with the control group for tests with a continuous score and results below the maximum possible score on the MoCA subtests. Two factors may have caused us to overestimate the MCI prevalence. Firstly, our inclusion of several MoCA items, where impairment was defined as a result below the maximum. As the Norwegian normal population with similar education in the relevant age group miss on average 4 points on the MoCA, this was likely too strict[195]. Secondly, comparing the PD patients to the probably supra-normal control group could lead to overestimation of MCI. Potentially compensating for this is that with a mean MoCA score of 27.1[194] the PD patients likely also had supra-normal cognition as well as the fact that we choose to define impairment as a result two SD below the mean in the control group, rather than the 1.5 SD which is frequently used. The PD-MCI group had lower education, higher UPDRS-III scores and were older than PD without cognitive impairment and the control group. Thus the limitations listed above may have led to misclassification of older individuals or those with low education as PD-MCI. We found a somewhat surprisingly high prevalence of multiple domain MCI (93%), this is in line with others applying the MDS MCI criteria, between 65 – 93% multidomain MCI have been reported[191].

5.1.2 CSF analysis

CSF marker analysis in dementia has been hindered by pre-analytical and analytical variation. Abeta42 is especially challenging, as it is a lipophilic protein and binds to surfaces in a non-specific manner[7]. The Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry has published an update where pre-analytical and analytical factors are extensively reviewed, in addition to the work being done to overcome these obstacles[7].

Much of this evidence has been gathered during the last decade, after the CSF collection in the Demvest project started in 2005. A standardized lumbar puncture and CSF handling procedure was developed and followed in Demvest. However, not all

recommendations in later guidelines were followed: we did not distribute tubes to the different centres, so different polypropylene tubes were used prior to shipping to the central storage, the CSF was frozen in smaller aliquots than are now recommended, we failed to fill tubes to 75 % of the volume after aliquoting and we labelled tubes manually, not with bar codes[7]. ELISA procedures were manual, relying on manual pipetting to a 96 well plate. Fully-automated assays have shown more stable measurements[7]. This might have influenced especially abeta42 concentrations and made our analysis less reliable. Most likely these factors have caused random errors, thus decreasing the strengths of our associations, but systematic errors cannot be ruled out.

For abeta42 analysis, we used an ELISA assay from Biosource, while t-tau and p-tau181 were analyzed with Innotest ELISAs (see Methods section 3.4.1). The Demvest samples were reanalyzed at Sahlgrenska University Hospital, Mölndalen as part of a multicenter study with Innotest total tau, Innotest p-tau181 and Innotest β-amyloid(1-42) ELISAs[196]. Correlations were good for total tau and p-tau181, with Spearman`s rho = 0.911 and 0.900 respectively ($p<0.001$ and $p<0.001$). However, the abeta42 results were less satisfactory, with rho 0.427 ($p=0.002$). Thus our t-tau and p-tau181 results are comparable to other studies, the abeta42 results less so.

The PPMI was designed years later and carefully planned to minimize pre-analytical variation. All centres received kits with all the material required for lumbar puncture and CSF handling. The “Biospecimen Collection, Processing, and shipment Manual” (see ppmi-info.org) describes the CSF handling in detail. A training video for correct CSF processing and aliquoting was made. However, it was a limitation that not all patients and controls had available CSF haemoglobin concentrations at the time of data download. But as described in the methods section, we took precautions to exclude cases with high α-synuclein values due to blood contamination.

5.1.3 Neuropathology

Brain dissection, tissue preparation, staining, immunohistochemistry and staging of pathology for paper III were done according to international consensus protocols, see the methods section. Not being a neuropathologist, I will not discuss the methods used further. However, some steps of the neuropathological diagnostic process warrant a comment. Many patients had several pathologies present at autopsy. Both the 2005[61] and 2017 DLB[62] criteria acknowledge that many patients have concomitant Lewy body and AD pathology, and provide the likelihood of a clinical DLB syndrome for different combinations of Lewy body and AD pathology (see Introduction section 1.4.3). In Paper III we chose to classify patients with a neuropathological "intermediate" or "high" likelihood of the DLB syndrome as DLB[62]. If only those with "high" likelihood had been classified as DLB, this would have increased sensitivity to a 100% for a clinical diagnosis of DLB; as all of the eight "high" likelihood cases had a clinical diagnosis of PDD or DLB. But decreased specificity to 83%, as six cases with a clinical DLB diagnosis had neuropathological "intermediate" likelihood. Also, two PDD cases did not fulfill criteria for "high" likelihood of DLB.

5.1.4 Statistical analysis

The sample for paper I contained only 32 patients, thus there is a clear risk for type II errors, there may have been real correlations that we were unable to detect due to low power. A limitation to the statistical analysis in Paper I was unadjusted correlations. T-tau increases with age in healthy individuals[197, 198] and memory clinic patients[199], while p-tau181 levels are stable[198, 199]. Whilst the prevalence of apathy has been found to increase with age in non-demented elderly[200, 201], this is not the case in AD patients[38]. Thus age is unlikely to have an effect on our results. Apathy has on the other hand been found to be associated with the level of education and dementia severity, perhaps as explained by lower education contributing to more severe cognitive impairment[38]. Thus, ideally we should have adjusted for education and dementia severity, but multivariate analysis was not possible in this small sample. However, the risk of bias

due to dementia severity was reduced by our inclusion criteria; all patients had mild dementia.

The large sample size is a major advantage for paper II. Limitations to the generalizability of results of paper II include that the standardized beta values were relatively small and the fact that we did not adjust for multiple testing.

5.2 Discussion of results

5.2.1 Associations between neuropsychiatric symptoms and biomarkers

Apathy

We explored the correlation between neuropsychiatric symptoms and CSF biomarkers in early AD, both out of interest in the neuropathological underpinnings of these symptoms, and because of reports of an association between neuropsychiatric symptoms and dementia progression[38, 202] and conversion from MCI to dementia[203]. Our main finding was a correlation between apathy and t-tau and p-tau181 concentrations in CSF. Few other studies have explored this. Contrary to our findings, t-tau and p-tau181 were not associated with severity of apathy at baseline, nor its development over time in a cohort of normal controls, MCI and AD patients[54]. This discrepancy may be explained by different patient groups and the use of a questionnaire version of the NPI to assess apathy in the other cohort[204]. We used the Starkstein Apathy Scale[164], which maps more aspects of apathy and is self-reported while the NPI is based on reports from a carer. In the Demvest cohort there were no associations between apathy and t-tau and p-tau when apathy was measured with the NPI apathy item (Spearman´s rho 0.062, p=0.756 and Spearman´s rho -0.16, p=0.935 respectively), thus supporting the theory that a specific, self-reported instrument measures something separate from a single NPI item.

Apathy is a symptom of vital importance for dementia patients, it is a strong predictor of ability to perform instrumental activities of daily living in MCI and AD patients[205],

and thus of the patient`s ability to live independently. Apathy is likely the most common neuropsychiatric symptom in dementia[38, 40, 206], reported to have both high incidence over time and high persistence[40]. In the Demvest study, apathy was the most prevalent neuropsychiatric symptom, increasing in AD patients over time[202]. Thus apathy is both common and persistent in AD, this adds weight to the theory that apathy may be linked to core AD pathology. Our findings of an association between increased p-tau181 and t-tau in CSF and apathy may suggest a role for neurofibrillary tangle pathology in its pathogenesis. The increased t-tau also concentration also suggests that neurodegeneration might be involved, as t-tau concentrations likely reflect the intensity of neuronal damage[7].

Prefrontal regions and the anterior cingulate are frequently associated with apathy in both structural and functional imaging studies of AD and MCI patients[207]. The anterior cingulate is associated with cerebral processes underlying goal-directed behaviour involving emotion, initiative and cognitive interest, and prefrontal regions are important for planning and strategic thinking[207]. This has led to theories that apathy in AD might be caused by dysfunction in these structures and/or circuits involving these regions[207]. Cholinergic and dopaminergic denervation have also been linked to apathy[208], and the theory that dopaminergic dysfunction is involved has been supported by a few randomized controlled studies finding that methylphenidate reduces apathy in dementia[208].

As the neurofibrillary tangle pathology starts in the transentorhinal cortex, frontal regions and the cingulate are first affected at later stages[18]. However, our findings of an association between the CSF markers of neurofibrillary tangle pathology and apathy are in line with two neuropathological studies reporting a correlation between neurofibrillary tangle load in the anterior cingulate region and apathy scores[209, 210], thus integrating our hypothesis with the finding from imaging studies of anterior cingulate involvement.

Depression

Our finding of no associations between CSF markers and depression in the newly diagnosed AD patients has since been replicated in AD[211] and MCI patients (mainly amnestic MCI)[212]. In line with our findings of no association between the CSF marker of beta-amyloid pathology and depression, no correlation was found between current depressive symptoms and cortical beta-amyloid depositions quantified with ^{18}F-florbetapir PET in MCI and AD patients[49]. Contrary to this, an autopsy study found higher levels of both amyloid plaques and neurofibrillary tangles in the hippocampus of AD patients with depression, compared to those without a history of depression[213]. Patients with depression also had a more rapid progression[213]. A higher density of beta-amyloid depositions assessed with ^{18}F-florbetapir have been found in the frontal cortex of amnestic MCI patients with a lifetime history of depression compared to their counterparts without previous depression[214], and in multiple brain regions in persons with a lifetime history of depression compared to those without previous depression[215]. Thus, whilst the evidence suggests an association between lifetime depression and beta-amyloid pathology, the same has not been shown for current depression in AD. Depression is an AD risk factor[1], more research is needed to ascertain whether the mechanism underlying this is depression driving beta-amyloid accumulations, as well as other possible mechanisms such as hypercortisolemia[216].

Psychosis

In contrast to our findings of no associations between psychosis and CSF markers, another study reported that CSF t-tau, but not p-tau181, was elevated in AD patients with psychosis at baseline or who developed psychosis over a 36-month period compared to those without psychosis[217]. Elevated t-tau alone, however, likely represents neuronal damage in general, and is not specific for AD[7]. The diverging results could be explained by larger sample size, they included 200 AD patients, and the longitudinal design, as psychosis is more common in moderate than mild dementia[1]. We included the agitation item of the NPI[165] in our definition of psychosis. In a study where a comprehensive assessment for agitation was included, agitation correlated with CSF t-tau and p-tau181 in dementia[218]. In 728 patients with a neuropathological AD diagnosis, no association were found between the severity of

amyloid plaque or neurofibrillary tangle pathology and psychosis (hallucinations and/or delusions) present at any time assessed using the NPI[219]. Thus our findings of no association between AD CSF markers and psychosis are mostly in line with other findings. However, comorbid Lewy body pathology, subcortical white matter lesions and vascular risk factors smoking, hypertension, diabetes and amyloid angiopathy are significantly associated with psychosis[219, 220].

A broader perspective on neuropsychiatric symptoms

Although neurobiological mechanisms likely play a role for neuropsychiatric symptoms, psychological, social, environmental and other mechanisms also contribute. With increasing dementia severity, the individual's ability to understand his or her surroundings, and communicate thoughts and needs are gradually lost. Psychological theoretical frameworks focus on triggers and reinforcements of the unwanted behaviour, on behaviour as a way to communicate unmet needs, and on symptoms as a reaction to an environment with too many stressors[221]. Both medical, environmental and caregiver factors affect neuropsychiatric symptoms[221].

5.2.2 CSF biomarkers and cognitive impairment in PD

Associations between CSF abeta42, t-tau and p-tau181 and cognition

As mentioned in the introduction, amyloid plaques and neurofibrillary tangle pathology are associated with cognitive impairment and dementia in PD[5, 222]. Our finding of decreased CSF abeta42 in the PD-MCI group after adjustment for covariates is in line with previous findings of decreased abeta42 in PD with cognitive impairment and dementia[223, 224]. Previous studies have found that decreased CSF abeta42 concentrations correlate with memory impairment in PD[187, 189, 225, 226]. Our study did not reproduce this, we found no association between decreased abeta42 and impairment of the memory domain. Discrepancies between our study and the previous publications might be due to differences in patient characteristics; the PPMI patients were younger and less cognitively impaired, and all were unmedicated[187, 189, 225, 226]. Two of the previous studies included PD patients with disease duration of several years[225, 226]. Decreased abeta42 in early PD predicts faster cognitive decline in

longitudinal studies[73, 74, 224, 227], including in the PPMI-cohort[228]. The same has been shown in DLB[131]. Thus, our findings support results from previous publications; decreased CSF abeta42 is associated with cognitive impairment in PD.

We found decreased t-tau and p-tau181 in PD patients compared to controls, but no association between t-tau or p-tau181 concentrations and cognition. Results regarding the relationship between t-tau and p-tau181 in CSF and cognition in PD are conflicting, some report that increased t-tau and p-tau concentrations are associated with cognitive impairment in PD[229-231] and also motor progression[232]. Contrary to this, others have reported decreased t-tau and p-tau181 concentrations in PD-MCI compared with PD with normal cognition[187] and PD-MCI compared to controls[233]. In the PPMI cohort increased total tau but not p-tau181 was associated with lower total MoCA score after two years of follow-up[228]. As reviewed by Lin et al, most studies find no association between CSF t-tau and p-tau concentrations and cognition[223]. One possible explanation for the discrepancies could be PD duration and severity; evidence for the impact of neurofibrillary tangle pathology on cognition come from autopsy studies naturally including mostly patients with long disease duration. One study found that t-tau and p-tau181 concentrations are higher in older patients with PDD than in younger patients with PD and normal cognition[231], another that while there was no link between p-tau181 concentrations and cognitive decline in very early PD, increased p-tau181 was associated with faster cognitive decline after initiation of dopaminergic treatment[234]. Thus, increased t-tau and p-tau181 might be markers for extensive AD pathology in PD, this is supported by findings from a study of autopsy verified LBD cases – those with an antemortem lower abeta42 and higher t-tau and p-tau181 concentrations had more AD pathology at autopsy[235]. In conclusion, although there is evidence for the importance of neurofibrillary tangle pathology on cognition and prognosis in PD, our finding of no association between cognition and increased CSF markers of neurofibrillary tangle pathology in early PD suggest this is less important for early cognitive impairment. The neurobiological mechanisms for our finding of decreased the t-tau and p-tau concentrations in PD compared to controls are unclear, and an important topic for further research.

CSF α-synuclein and cognition

Since α-synuclein is the key protein in the development of LBD, it is tempting to explore this as a potential CSF biomarker. We found that reduced α-synuclein in CSF was significantly associated with impaired global cognition assessed with the composite cognition factor, and with impairment of the executive/attention domain in early PD.

Table 8: Studies analyzing the association between CSF α-synuclein concentrations and cognition in PD

Author and year	Number and age of PD patients	Cognition (result screening test)	α-synuclein concentration and assay	Disease duration, and dopaminergic treatment	Results
Yarnall AJ. 2014[187]	N=67 Mean age 65.	Mean MMSE 29*.	Mean 110 pg/ml. Immunoassay on Meso Scale Discovery electro-chemilyminescence platform.	Mean 6 months*. Mean LEDD 178 mg.	No significant associations between α-syn and cognition.
Stewart T. 2014[236]	Phase 1: N=350, phase 2: N=266. Phase 1: mean age 61, phase 2: mean age 63.	MMSE Phase 1: mean 29, phase 2: mean 29.	Phase 1 mean 0.63 ng/ml, phase 2 0.47 ng/ml. Analyzed with Luminex assay developed by the authors.	Phase 1 (prior to treatment with levodopa): mean 2.1 years. Phase 2 (after initiation of levodopa treatment) mean 3.8 years. Phase 1: unkown proportion on deprenyl (selegelin). Phase 2: 100% on levodopa, no information about LEDD.	Cross-sectional results: Phase 1: decreased α-syn was associated with decreased delayed recall. Phase 2: increased α-syn associated with decreased total recall. Higher α-syn at the beginning of phase 2 predicted faster decline in total and delayed recall and visuospatial working memory.
Buddhala C. 2014[239]	N=77 Median age 67.	CDR < 1	Median 1853 pg/ml. Covance α-syn ELISA.	Median 4.9 years. Median LEDD 811 mg.	Cognitive domains were created from neuropsychological test battery. No significant associations found with α-syn.

Hall S. 2015[132]	N=42 Median age 68.	MMSE median 29.	Median 50.5 ng/L. Analyzed with a Luminex assay developed by the authors which concomitantly measures abeta42, t-tau, p-tau181 and α-syn.	Median 7 years, median LEDD 648 mg.	Increased α-syn associated with worsening of cognitive processing speed over two years.
Skogseth R. 2015 (paper II). $	N=414 Mean age 61.	MoCA Median 28.	Median 1710 pg/ml. Covance α-syn ELISA.	Median 4 months since PD diagnosis. No use of dopaminergic treatment.	Association between decreased global cognition and executive/attention function and decreased α-syn.
Stav A. 2015[225]	N=24 Mean age 65.	MMSE Mean 29.	Mean 276 ng/L. Meso Scale Discovery.	Mean disease duration from motor onset 2.5 years. Mean LEDD 385.2 mg.	No correlation between neuropsychological tests and α-syn.
Compta Y. 2015[237]	N=41. 21 PD no dementia median age 68. 20 PDD, median age 74.	MMSE: PD no dementia: median 28. MMSE PDD median 18.	PD no dementia: median 0.3877 ng/ml, PDD 0.453 ng/ml. ELISA from Invitrogen, with minor modifications.	PD no dementia: median 10 years of parkinsonism, PDD median 9 years of parkinsonism. No information about dopaminergic treatment.	CSF α-syn levels were lower in PD patients with impaired vs normal phonemic fluency. Decreased recognition (from Ray Auditory Verbal Learning Test) and language (Boston naming test) correlated with increased α-syn.
Kang J. 2016[180] $	N=412. Median age 62.	MoCA Median 28.	Median 1715 pg/ml. Covance α-syn ELISA	Median 4 months since PD diagnosis. No use of dopaminergic treatment.	Lower α-syn was significantly associated with impairment on several cognitive tests.
Majbour NK. 2016[238]	N=46. Median age 64.	MMSE Median 29.	PD 1.3 ng/ml. ELISA developed by the researchers.	Median 4 years. No information about dopaminergic treatment.	Inverse correlation between α-syn and MMSE. MMSE was only cognitive test.
Chiassineri D. 2017[133]	PD=54, PDD=20. Mean age PD=66, PDD 74.	Mean MMSE PD 28, PDD 19.	PD 1846 pg/ml, PDD 1382 pg/ml. ELISA developed by ADx NeuroSciences Ghent, Belgium).	No information.	No associations between MMSE results and α-syn. MMSE was the only cognitive test.
Goldman J. 2018[226]	N=115. Mean age 68.	MoCA Mean 27.	Mean 1466 pg/mL. ELISA assay from BioLegend.	Mean 8 years. LEDD mean 741 mg.	Only cognitive test MoCA, no significant associations with α-syn.

Only included PD-patients with CSF α-synuclein results are listed, some studies have also included controls and/or patients with other dementia diagnosis. Abbreviations: α-syn=α-synuclein. MMSE: Mini-mental state examination. MoCA: Montreal Cognitive Assessment. ELISA: Enzyme linked immunosorbent assay. CDR: The Clinical Dementia Rating[152]. LEDD: Levodopa equivalent daily dose.
***Mean MMSE, disease duration and levodopa-use are reported for the whole PD cohort (n=219), results are not given for the individuals who have donated CSF separately. $Both papers have analyzed the PPMI cohort.**

For an overview of studies exploring the association between CSF α-synuclein and cognition, see table 7. Briefly, cognitive impairment has been found to be associated with increased α-synuclein[132, 236-238], decreased α-synuclein[180, 236, 237] and without any relationship to CSF α-synuclein concentrations[133, 187, 225, 226, 239] in PD. Of the 12 studies summarized in the table 8, five included fewer than 50 patients, three between 50 – 100 patients, the largest studies were the ones by Stewart et al[236] with 350 patients, Goldman et al with 115 patients[226] and the studies based on the PPMI cohort; the study by Kang and coworkers[180] and my paper II.

The largest study apart from the PPMI, the DATA-TOP trial, included newly diagnosed PD patients from 1987 – 1988, thus samples were stored for two decades before analysis[236]. Originally this was a randomized control trial of deprenyl and α-tocopherol, with a phase one from inclusion to levodopa treatment was needed, and phase two after initiation of treatment, average follow up was 1.8 years. In phase one, low α-synuclein concentrations correlated significantly with impairment on a neuropsychological test of delayed recall. Higher CSF α-synuclein levels at the beginning of phase two were associated with faster cognitive decline over phase two[236]. This association between higher α-synuclein levels and faster cognitive decline was also found in another study of PD patients on dopaminergic treatment (median disease duration seven years)[132]. High CSF total α-synuclein concentrations correlated with impaired cognition (total MMSE score) in another study that likely included PD patients mainly on dopaminergic treatment (the proportion of patients receiving treatment was not stated, but the median disease duration was 4 years)[238]. In line with our findings of an association between reduced α-synuclein and impairment of executive function and attention, Compta and coworkers found that reduced α-synuclein was significantly associated with impaired phonemic fluency (testing executive function) in PD and PDD patients[237]. In the same study, increased CSF α-synuclein was associated with impairment of verbal memory and language, but of note, these associations were lost when t-tau concentrations were added as a covariate[237]. Of the studies exploring the relationship between cognition and α-synuclein, the PPMI is the largest, with well-characterized patients, and the study itself is designed for assessment of biomarkers.

Initiation of dopaminergic treatment replaces lost dopamine from those areas affected by primary dopaminergic deficits such as the putamen, however, areas that do not yet suffer from loss of dopamine may be overdosed[240]. As a result, dopaminergic treatment may improve cognitive functions dependent on frontostriatal circuits severely affected by dopaminergic deficits, but worsen others, such as learning based

on feedback and trial and error due to an overdose of dopamine[240]. Thus, if the frontal executive/attentional dysfunction we found to be associated with α-synuclein is affected by treatment, this could be one explanation for the discrepant findings. This would add to all the other differences between the studies that explore the association between cognition and α-synuclein; PD patients at different disease stages, cognition tested with methods ranging from simple screening instruments (MoCA or MMSE) to neuropsychological test batteries, and some studies including follow up while others are cross-sectional. CSF α-synuclein has also been found to be decreased in subjects with a postural instability/gait difficulty phenotype compared to cases with tremor dominant or indeterminate phenotypes[180, 226], thus clinical phenotype of the included patients could also play a role.

CSF α-synuclein as a biomarker

A number of studies have explored the utility of CSF α-synuclein as a biomarker for PD, but the results have been conflicting. Both pre-analytical and analytical factors probably contributed to the inconsistent results. Commercial assays for the analysis of α-synuclein in CSF have been available for a much shorter time than for abeta42, t-tau and p-tau181. The concentration of total CSF α-synuclein in PD patients in the studies listed in table 8 above range from 110 – 23 000 pg/ml. Different assays were used, and to date, there is no accepted standard method. Thus it is by no means certain that these studies have measured the same entity, the different antibodies used in the immunoassays might have bound to different α-synuclein species.

However, total α-synuclein might not be the most clinically important α-synuclein species or the most suitable biomarker. Several α-synuclein aggregates have been identified, including soluble oligomers consisting of 2 – 100 α-synuclein monomers[81]. Soluble oligomers might be the toxic agent in LBD[241], acting as seeds catalysing the transformation of α-synuclein monomers to insoluble fibrils[241, 242]. Increased oligomer concentrations have been found in brain homogenates of both DLB and PD patients[241]. CSF oligomer concentrations were higher in PD than in AD, PSP and controls[227, 238, 243], and the ratio between CSF oligomers / total -α-synuclein was able to separate PD

patients from controls with a sensitivity of 89% and a specificity of 91% in one study[243], and 82% and 64% in another[227]. Oligomeric α-synuclein concentration has also been found to correlate with the severity of motor symptoms in PD[238] and to increase in the same patients after two years[244]. When CSF from PD patients was added to recombinant α-synuclein, increasing PD severity caused more rapid aggregate formation[242]. More research is needed to determine α-synuclein oligomers potential as biomarkers and targets for treatment.

5.2.3 DLB diagnosis and neuropathology

Comorbid AD and LBD pathology and false negative patients

Our findings from the PPMI study are based on patients shortly after the first clinical presentation of neurodegenerative disease, while our neuropathological results are mostly patients with severe impairment. It is interesting that patients with both early and end-stage disease show evidence of comorbid AD and Lewy body pathology in cognitive impairment; newly diagnosed PD patients with MCI had lower CSF abeta42 concentrations than those with normal cognition and at autopsy ten of 31 patients with a neuropathological diagnosis of AD had some degree of Lewy body pathology and nearly all the 20 LBD patients had some degree of AD pathology. Decreased CSF abeta42 has been found to correlate with increased AD pathology at autopsy in LBD patients[235]. In addition to additive effects of comorbid pathologies, there are evidence for interactions at the molecular level[16, 245]. As my thesis shows, the co-occurrence of AD and LBD pathology is common and might prove to be an important factor in the neurobiology of neurodegenerative cognitive impairment, further research into comorbid pathology and protein interactions should be a priority.

Four patients were clinically diagnosed as AD but proved to have DLB at autopsy. All four were without DLB features at baseline, but in three the core features developed after two-three years of follow-up. The fact that all false negative DLB patients had severe AD pathology with Braak neurofibrillary tangle stage VI supports the notion that comorbid pathology influences the clinical phenotype.

None of the PDD but nine DLB patients had Braak neurofibrillary tangle stage VI. Thus four of the patients with diffuse neocortical Lewy body pathology and the most severe AD pathology were clinically classified as AD, 5 as LBD. One could argue that these are mixed AD-DLB cases, with a clinical phenotype reflecting both the Lewy body and AD pathology. So what differentiates these patients, with similar pathology but different clinical phenotypes? A neuropathological study of mixed AD-DLB patients found that patients that presented as clinical AD had higher neurofibrillary tangle load with a different anatomical distribution than those who presented with clinical DLB[246]. In those that presented as AD, the anatomical distribution of neurofibrillary tangles was similar to those in pure AD, whilst the cases that presented as DLB had less hippocampal neurofibrillary tangles[246]. This suggests the total burden of the different protein aggregates and anatomical distribution could play a role. However, it is also important to bear in mind that the neuropathology is the end stage, and the temporal development of pathology is unknown. Our results also highlight that the core DLB features can develop after the onset of dementia. In patients with autopsy verified DLB, only 22% had visual hallucinations, 26% had parkinsonism and 13% both at the first visit to a secondary care dementia clinic[247]. Thus the emergence of core DLB features in the first years after diagnosis should warrant consideration of whether a revision of the clinical diagnosis is necessary.

False positive patients

Two of the four patients with a clinical DLB diagnosis but AD at autopsy, i.e. false positives, had visual hallucinations and severe impairment of vision due to age-related macular degeneration. Complex visual hallucinations can occur in persons with significant visual impairment in the absence of mental disease[110]. In autopsy-verified cases, the odds of LBD were 4-5 times greater than AD if hallucinations occurred during the first 5 years of dementia, but over the whole course of disease hallucinations were equally common in AD and LBD[109]. Misdiagnosis of AD patients with early visual hallucinations as DLB has also been reported by others, patients with neuropathologically confirmed AD were five times more likely to be misclassified as DLB if they had psychosis[248]. In our study, the two patients with visual impairment

fall into this category, with both having severe AD at pathology (Braak neurofibrillary tangle stage VI) without any Lewy body pathology. In LBD, hallucinations have been found to correlate with limbic Lewy body density, while in AD early visual hallucinations were associated with greater density of limbic and cortical neurofibrillary tangles[109]. Interestingly, our third false positive patient had limbic Lewy body pathology in addition to severe AD. The association between limbic Lewy bodies and hallucinations has also been found in PD patients without dementia[249].

All the false positive patients had gradually increasing UPDRS-III scores, but of note two of them had received antipsychotics. One of these two patients was treated with risperidone from shortly after diagnosis right up until his death, the dose was increased over the years. The DLB criteria require that parkinsonism is spontaneous, not due to stroke or antidopaminergic medication[62]. Whilst these particular patients fulfilled the DLB criteria independent of parkinsonism due to visual hallucinations and fluctuations, however, use of antipsychotics might lead to misinterpreting drug-induced parkinsonism for a DLB feature and misdiagnosis.

The accuracy of clinical DLB diagnoses

In our prospective cohort study, with annual follow up until death, 16 of 20 patients with neuropathological DLB were correctly identified, resulting in a sensitivity of 73% and a specificity of 93% for the 2005 DLB consortium clinical criteria against autopsy. Few other prospective studies have examined the accuracy of the DLB clinical 2005 diagnostic criteria.

In a systematic review of the literature until October 2016, Rizzo and co-workers found a pooled sensitivity, specificity and accuracy of the 1996 criteria in early dementia of 19%, 95% and 78% respectively[250]. Their systematic review included only two studies using the clinical 2005 criteria, and those two studies included patients with late-stage disease – in both studies diagnosis were re-evaluated until death[250]. See Table 9 for an overview of important publications on diagnostic accuracy in DLB after 2016. The table also includes a publication by Nelson and co-workers[8] with clinical

diagnosis without further specification which was not included in Rizzo and co-workers review.

Table 9. Sensitivity and specificity of clinical DLB versus autopsy

Author and publication year	Patients and neuropathological diagnoses	DLB criteria	Methods	Results
Nelson PT. 2009[8]	N=2861. DLB 162, mixed AD and DLB 248, pure AD 1455, no AD or DLB 996.	Clinical DLB diagnosis, patients included after 2000, study from 2009.	Patients from 32 academic medical centres. Inclusion and exclusion criteria made to include patients with AD and LBD pathology.	Sensitivity 32%, specificity 98%.
Thomas AJ. 2016[136]	N=55. DLB 30, AD 22.	Clinical DLB diagnosis.	Patients were assessed annually "until they could no longer comply", and final assessment prior to death recorded for this analysis.	Sensitivity 87%, specificity 72%.
Fujishiro H. 2008[114]	N=76. DLB 46.	Probable DLB (2005 criteria).	Cases with intermediate or high neuropathological likelihood of DLB classified as DLB.	Sensitivity 87%, specificity 90%*.
Ferman TJ. 2011[116]	N=234. DLB 98, 136 other dementia.	Clinical 2005 DLB criteria.	The core features of DLB were considered present if they developed at any time. Cases with neuropathology consistent with intermediate or high likelihood of the clinical DLB syndrome were classified as DLB.	DLB was classified as ≥ two of visual hallucinations, parkinsonism, fluctuations and RBD. Sensitivity 88%, specificity 73%.
Selvackdunco S. 2019[9]	N=180; controls + unselected dementia cohort. DLB 2, mixed AD-DLB 28.	Clinical diagnosis, not stated criteria. Included patients died between 2009 and 2016.	Patients with clinical follow up, not stated for how long. Clinical diagnosis from routine clinical work-ups, majority specialist clinics.	When controls were excluded (n=55), sensitivity for combination of clinical DLB and mixed AD-DLB against neuropathological moderate-high likelihood of DLB was 20%, specificity 98%*.

*Limitation: Calculated by me, not in the original publication. Abbreviations: RBD: REM sleep behaviour disorder.

In their review, Rizzo and co-workers conclude that the clinical DLB criteria have become more sensitive and less specific over time[250]. The decrease of specificity is based mainly on the papers by Fujishiro[114] and Ferman[116] (see Table 9). However, the approach in these two papers makes their results unfit for comparison with standard dementia diagnostics. In both papers, diagnoses were re-evaluated yearly until death. In the paper by Ferman and co-workers core DLB features were considered present if they developed at any time-point[116]. But, as their study also demonstrates, the core DLB features of visual hallucinations, fluctuations and parkinsonism lose their specificity for DLB in severe dementia. In the patients with other dementia diseases than DLB at autopsy, 40% had fluctuations, 24% had visual hallucinations and 38% had parkinsonism at any time from diagnosis to death. But the timing was different,

50% of DLB patients had fluctuations at baseline compared to 29% in the other dementia group, and time from estimated dementia onset to visual hallucinations and parkinsonism was an average of two years in the DLB group compared to 5 years in the other dementia patients[116]. This was also seen in the paper by Nelson and co-workers (see Table 9), in patients with relatively severe dementia (mean MMSE 11.4), the core DLB features parkinsonism, visual hallucinations and fluctuations did not predict neuropathological LBD[8].

As study clinicians had a particular emphasis on DLB, and structured rating scales for all core DLB symptoms were used, it is likely that we identified more patients with DLB than in standard clinical routine. In support of this, we observed differences between our recruiting centres. The majority of patients were recruited from three outpatient clinics. Notably, three of the four patients with a false negative DLB diagnosis were recruited from one centre, where there were no clinicians with a special clinical or research interest in DLB. If the 16 patients from this centre were excluded, sensitivity and specificity of a clinical DLB diagnosis would increase to 90% and 100%, respectively. Our clinical diagnostic procedure was based on expert consensus, and none of the clinicians in this centre participated in the process. Our results demonstrate the limitations of a diagnostic procedure based solely on the assessment of standardized measures and case notes without direct contact with the patient. In order to be useful, the DLB criteria must be sufficiently robust to be used by clinicians without expert knowledge on DLB. Our results also indicate that the 2005 criteria perform very well when applied by expert clinicians focusing particularly on DLB, but lack sufficient sensitivity outside such centres.

Nelson and co-workers found a sensitivity of 32% and a specificity of 98% for patients diagnosed in clinical routine in a large study published in 2009 (see table 9)[8]. Unfortunately, the results by Selvackdunco and coworkers from 2019 indicate that sensitivity in clinical routine has not improved over the last decade, as they report a sensitivity of 20% and specificity 98%[9]. It remains to be seen whether the changes made in the clinical DLB criteria published in 2017 by DLB Consortium will improve

accuracy. The threshold for parkinsonism was lowered, now only one of the cardinal features of parkinsonism are required, this could increase sensitivity. However, it also has the potential to decrease specificity and increase the number of false positives, as motor symptoms can occur in other dementia[116] and cognitive impairment during the neurological examination and musculoskeletal conditions might be misinterpreted as parkinsonism[62].

In conclusion, our findings as well as those by Nelson[8] and Selvackadunco[9], indicate that the clinical DLB criteria are not yet sensitive enough, however, the specificity is satisfactory. Evidence to date does not support a decrease in the specificity of clinical DLB diagnosis in mild dementia with the introduction of the 2005 DLB criteria, however the clinical DLB criteria are unsuitable for differential diagnosis in severe dementia. However, as we have shown, the core features may develop during the first years after dementia onset in DLB. Future research must ascertain the accuracy of the clinical 2017 DLB criteria, and clarify what time-frame between onset of cognitive symptoms and core features are consistent and inconsistent with DLB. The disappointing sensitivity in clinical routine DLB diagnostics also demonstrates the need for biomarkers. Fortunately, both polysomnography and DAT imaging have been found to increase the accuracy of clinical DLB diagnosis in autopsy verified cases[116, 136]. Increased clinical use would probably increase accuracy. Unfortunately, their use is limited by availability and costs. There is a clear need for more easily available biomarkers to aid clinical DLB diagnosis, and EEG is a promising candidate[251, 252].

6. Conclusions

6.1 Paper I

The main findings in paper I were that CSF t-tau and p-tau181 significantly correlated with apathy in early AD. However, no correlations was found between depression and psychosis and any markers. The associations found suggests a link between neurofibrillary tangle pathology and apathy. Results must, however, be interpreted with caution as paper I have limitations, the most important being the small patient sample. The neuropathological underpinnings of neuropsychiatric symptoms in AD are not yet fully understood, but most likely heterogeneous with a combination of psychosocial, individual and neuropathological factors.

6.2 Paper II

The main findings of paper II were the correlation between decreased α-synuclein concentrations in CSF and impairment of both global cognitive function and the executive function/attention cognitive domain and decreased abeta42 concentrations in CSF from PD-MCI patients compared to PD with normal cognition and healthy controls. Reduced α-synuclein was also associated at trend level with impairment of both memory and visuospatial domains.

6.3 Paper III

The main findings from paper III were that the clinical criteria for probable DLB had a sensitivity, specificity, positive and negative predictive values of 73%, 93%, 79% and 90% respectively, compared to pathological diagnosis. All PDD patients were correctly classified. Three patients were false positive, with a clinical DLB diagnosis but neuropathological AD. Two of the three had no LBD pathology, but impaired vision and visual hallucinations at baseline, highlighting the importance of taking visual impairment into account when patients present with hallucinations.

Four neuropathological DLB cases were given a clinical AD diagnosis. None had core DLB features at baseline, but these developed two–three years into the study in three of these patients. All of them had severe AD at autopsy, with Braak neurofibrillary tangle stage VI, and could thus be considered mixed AD-DLB cases, highlighting the influence of comorbid pathology on the clinical phenotype. Our results show that core DLB features can develop after diagnosis and that the sensitivity of a clinical DLB diagnosis is probably still suboptimal, especially outside centres with a particular interest in DLB.

7. Future perspectives and implications

7.1 Neuropsychiatric symptoms are key features of dementia

We conducted study I out of a conviction that neuropsychiatric symptoms are key factors in dementia, and thus must be linked to neuropathology. The importance of neuropsychiatric symptoms has since the publication of our paper been confirmed by other studies. Late-life neuropsychiatric symptoms such as affective symptoms, apathy, and socially inappropriate behaviour increase the risk of dementia more than MCI[253], increase the risk of progression from MCI to dementia[254, 255] and decrease the likelihood of reversion from MCI to normal cognition[254]. Neuropsychiatric symptoms are also associated with poor clinical outcomes[43, 256-258], increased mortality[43, 257] and dementia progression[43, 257, 258], and there are few treatment options. Understanding the mechanisms underlying these symptoms is therefore of the highest importance in order to develop new and better treatment strategies for these key features of dementia.

Future research is needed to clarify to what degree there is a link between particular pathologies and neuropsychiatric symptoms, and to what degree the same phenotype can be explained by several kinds of pathology. For instance, visual hallucinations in AD have been found to correlate with both neurofibrillary tangles, vascular lesions and Lewy body pathology[219, 259], hallucinations are also associated with cerebral amyloid angiopathy[220].

7.2 α-synuclein as a Lewy body pathology biomarker

Studies exploring the association between cognition and CSF total α-synuclein have reported discrepant findings. Total α-synuclein concentrations are highly variable between studies, with significant overlap between LBD and controls. As a biomarker CSF total α-synuclein is immature and future research might prove it to be unsuitable.

More research is needed to determine whether α-synuclein oligomers might prove to be valuable LBD biomarkers or targets for treatment. One could argue that today's LBD biomarkers such as DAT imaging, polysomnography and low uptake on ^{123}iodine-MIBG myocardial scintigraphy are markers of the *consequences* of the α-synuclein and Lewy body pathology, rather than of the pathology itself. There is a clear need for more direct biomarkers of LBD pathology.

7.3 Diagnosing dementia

We reported that the 2005 clinical DLB criteria[61] had suboptimal sensitivity. Research based on the 2017 criteria[62] will show if the changes made, such as lowering the parkinsonism threshold and inclusion of RBD as a core criterion, is sufficient to increase sensitivity without unacceptable effects on specificity.

The main theme of my thesis has been associations between clinical symptoms and neuropathology. Both paper II and III add to overwhelming evidence that concomitant pathologies are more the rule than the exception in neurodegenerative cognitive impairment. The relationship and potential interaction between pathologies must be clarified in the years to come.

Paper III demonstrates that detailed characterization of the clinical phenotype is a powerful predictor of neuropathological lesions. However, it also shows that a purely clinical approach has major limitations in mixed AD and LBD cases. More severe AD pathology increased the risk of patients with LBD pathology being classified as AD, comorbid AD pathology in LBD also influence survival and progression of dementia[5, 260]. An efficient treatment for one of the proteinopathies will make it imperative to detect the pathology actually present, and in mixed cases and likely prodromal cases this will require the use of biomarkers. It remains to be seen whether increased knowledge from biomarkers about the neurobiological process in the individual patients will change the dementia diagnostics paradigm that dominates clinical

medicine today; that these neurodegenerative diseases are diagnosed only after the emergence of the dementia syndrome and mainly one diagnosis per patients.

Both CSF biomarkers and imaging will likely be important to detect neuropathology in vivo. Work is being done to standardize the analysis of CSF t-tau, p-tau181 and abeta42 to decrease the variation that hampers efficient use of the markers today[7]. More studies with both careful clinical evaluation and follow-up, biomarker analysis and neuropathological evaluation are necessary to clearly establish the association between clinical presentation, biomarkers and the contribution of different neuropathological lesions.

7.4 Clinical implications

Clinical implications of my thesis include that cognitive impairment is present in a significant fraction of patients with early PD, highlighting that PD is not simply a movement disorder – not even at disease presentation. This should be taken into account in patient care.

Also, the risk of misdiagnosing patients with early DLB as AD is still substantial. Even when we applied structured rating scales for all core DLB features and referred a subsample to DAT imaging, we only reached a sensitivity of 73% for DLB. Selvackdunco and coworkers[9] findings corresponds to a sensitivity of only 20% for clinical routine DLB diagnosis in the United Kingdom at approximately the same time as our Demvest study. The DLB Consortium recommends that structured rating scales for fluctuations and RBD are used, DAT imaging in cases where parkinsonism is clinically uncertain and polysomnography if there is a doubt as to whether a sleep disturbance is RBD. In addition to this, diagnoses should be re-evaluated if the core DLB features emerge in the first years after dementia onset.

8. Literature

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