Cerebrospinal fluid biomarkers in dementia with Lewy bodies

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CHAPTER 1

General Introduction

INTRODUCTION

Improvements in the quality of health care and social circumstances have led to a remarkable increase in average life expectancy and, as a consequence, to a higher incidence and prevalence of age-related diseases. Neurodegenerative diseases leading to dementia are among the largest health care challenges of our century. Currently, dementia affects approximately 280.000 people in the Netherlands [1] and 50 million people worldwide.[2] It is estimated that these numbers will triple to 152 million cases worldwide by 2050.[2] Dementia is a clinical syndrome characterized by progressive cognitive decline with subsequent impairment in daily functioning.[3] It can be caused by several neurodegenerative diseases, with Alzheimer's disease being the most common cause. Other frequent causes of dementia are dementia with Lewy bodies, vascular dementia, and frontotemporal dementia. Despite numerous efforts, there are currently no curative treatments against these neurodegenerative diseases. Early and accurate diagnosis will most likely benefit clinical management and the development of future therapeutic interventions. This prospect emphasizes the urgent need to develop biomarkers to aid in diagnostic workup of neurodegenerative diseases. The focus of this thesis is on cerebrospinal fluid biomarkers for dementia with Lewy bodies.

Dementia with Lewy bodies: pathological hallmarks and clinical presentation

The neurologist Fritz Heinrich Lewy provided the first description of intracellular inclusions of proteins within the neuron of the cerebral cortex of patients with Parkinson's disease (PD) in 1912.[4] A few years later the Russian neuropathologist Konstantin Nikolaevich Tretiakoff identified the presence of similar protein inclusions in the substantia nigra of PD patients and named these inclusions 'Lewy bodies'.[5] Not until 1976, Kenji Kosaka and colleagues described the first post-mortem case of presenile dementia with Lewy body pathology.[6, 7] Several decades later, in 1996, the term 'Dementia with Lewy bodies' (DLB) was introduced.[8]

Epidemiology

DLB is a common neurodegenerative disease in the elderly. DLB accounts for 4.2% of dementia patients clinically diagnosed in primary care and 7.5% in the secondary care.[9] However, prevalence estimates from autopsy studies lay between 15-20%,[10] suggesting that many DLB cases remain unrecognized in clinical practice. The mean age of onset is 75 years, with a range from 50 to 80 years, and there is a male predominance.[11]

Pathological hallmarks

The pathological hallmark of DLB are Lewy bodies and Lewy neurites. Lewy bodies are eosinophilic intraneural inclusions composed of multiple proteins and lipids, with aggregates of the protein α-synuclein as the main constituent.[12] The precise biological function of α-synuclein remains to be elucidated, but it is probably involved in synaptic transmission.[13] Under pathologic conditions, conformational changes convert α-synuclein from its natively unfolded state to form oligomers and protofibrils, and ultimately β-sheet fibrils that aggregate as Lewy bodies.[14] Lewy body pathology can also be detected in the brains of patients with PD.[12] In PD, the topographical distribution pattern, of α-synuclein propagates from brain stem, to the substantia nigra, and further to the neocortex[15] while in DLB α-synuclein pathology may initially start in limbic and/or neocortical areas.[16, 17] The anatomical distribution and load of α-synuclein pathology is considered to be linked to the clinical symptoms seen in both disorders. For example, Lewy bodies in the brainstem have been associated with motor symptoms, whereas cognitive impairment has been attributed to Lewy bodies in the limbic and neocortical areas.

Lewy body pathology in DLB is thought to cause a progressive loss of structure and functions of the neuron, eventually leading to neural cell death.[18] Growing evidence suggests that not just intracellular aggregation of α-synuclein, but also small aggregates of α-synuclein oligomers have pathological significance.[19] Toxic α-synuclein aggregates at presynaptic terminals affect several steps of synaptic function, including maintenance of synaptic vesicle pools, vesicular transport/trafficking, and docking, priming a fusion of the synaptic vesicle pool at the synaptic cleft. Moreover, synaptic alterations are accompanied by abnormalities in neurotransmitter release.[20-24] Neurotransmitter deficiencies with reductions in acetylcholine, dopamine and norepinephrine, are evident in DLB.[25] Synaptic dysfunction and the resulting neurotransmitter deficiencies might precede neuronal degeneration in DLB, and may be more closely related to cognitive decline than pathological hallmarks such as Lewy bodies [26] but, like Lewy body pathology this cannot yet be measured *in vivo*.

Although primarily classified as a synucleinopathy, DLB is often accompanied by other age-related neurodegenerative pathologies. In particular, AD-related pathology, i.e. extracellular amyloid-β plaques and intracellular neurofibrillary tangles, is present in approximately 50% of all DLB patients upon postmortem evaluation.[27, 28] The co-occurrence of Lewy body pathology and AD pathology suggest a potential synergistic relationship between these two types of neuropathologies. Animal and cellular model studies have shown that amyloid-β, abnormal phosphorylated Tau, and α-synuclein

synergistically interact to promote their mutual aggregation.[29-31] These interactions highlight the interface between these misfolded proteins and may point towards shared biological mechanisms and overlapping pathology. In this line, APOE-ε4, the strongest known genetic risk factor for AD, has been shown to independently contribute to both α-synuclein pathology and AD-pathology in DLB.[32, 33] The importance of AD pathology in DLB is not yet clear. Evidence from clinicopathological studies suggests that patients with both DLB and AD pathology exhibit a more aggressive disease course and more pronounced cognitive dysfunction compared to pure AD or DLB.[27, 34, 35] Furthermore, the presence of AD pathology may reduce the likelihood of expression of the typical DLB phenotype (see below).[35] Understanding of the contribution of AD pathology to DLB is important, particularly as the field is moving forward to develop disease-modifying treatments designed to target amyloid-β plaques and intracellular neurofibrillary tangles.

Clinical phenotype

DLB is a complex disease that influences daily and social living activities resulting in serious impairment of quality of life. The clinical features of DLB include a combination of cognitive, motor, neuropsychiatric and autonomic symptoms. Patients with DLB show a wide spectrum of cognitive symptoms, with largest deficits in the attention, executive function and visuospatial domains. Memory function is usually preserved early in the disease course of DLB. Fluctuations in cognitive function and alertness have been observed in 50-75% of DLB patients. Compared with PD, motor symptoms are less prominent at onset, but during the disease course bradykinesia, postural instability and gait abnormalities are frequently present. Neuropsychiatric symptoms, especially visual hallucinations and apathy, and REM sleep behavior disorder (RBD) are regularly encountered in DLB and frequently present early in the disease course. Many DLB patients also suffer from autonomic failure (e.g. orthostatic hypotension, constipation and micturition problems).[11]

Dementia with Lewy bodies: a diagnostic challenge

A definite diagnosis can only be made postmortem, and the diagnosis of DLB ante mortem is foremost based on clinical features. The first diagnostic criteria for DLB were published in 1996. These criteria were revised in 2005 and 2017.[8, 36, 37] The central feature is dementia. Since the first formulation of the consensus criteria, the clinical features have been subdivided into either core or supportive features with the former being considered necessary to confer sufficient probability of underlying Lewy body pathology to make the diagnosis. Core clinical features include visual hallucinations, fluctuations in cognition, parkinsonism and RBD. Supportive clinical features of DLB

include, amongst others, sensitivity to neuroleptics, autonomic dysfunction, hyposmia, hallucinations in other modalities, delusions, apathy, and depression (see box 1).[36]

Box 1 | Diagnostic consensus criteria for DLB (McKeith et al., 2017)[36]

Diagnosis		Central feature	Core clinical features	Supportive clinical features	Indicative biomarkers	Supportive biomarkers
		Dementia Clinical syndrome characterized by progressive cognitive decline with subsequent impairment in daily functioning	**Recurrent visual hallucinations** **Fluctuating cognition** with pronounced variations in attention, alertness and concentration **Parkinsonism** (bradykinesia, rigidity, resting tremor) **Rapid eye movement sleep behavior disorder**	**Sensitivity to neuroleptics** **Repeated falls** **Syncope** **Autonomic dysfunction** **Hypersomnia** **Hyposmia** **Hallucinations in other modalities** **Psychiatric symptoms** (delusions, apathy, anxiety, depression)	**DAT-SPECT scan** Reduced dopamine uptake in basal ganglia **I-MIBG myocardial scinitigraphy** Low uptake **Polysomnography** Confirmation of REM slap without atonia	**MRI/CT-scan** preservation of medial temporal lobe **FDG-PET imaging** reduced occipital activity, cingulate island sign **EEG** Posterior slow-wave activity
Probable	Option 1	yes	≥ 2 features			
Probable	Option 2	yes	1 feature		≥ 1 biomarker	
Possible	Option 1	yes	1 feature			
Possible	Option 2	yes	0 features		≥ 1 biomarker	

Accurate diagnosis of DLB is important for adequate prognostication, clinical management and patient selection into clinical trials, however accurate diagnosis of DLB remains challenging. Symptoms may not be uniform between patients with DLB, even for core symptoms. For example, some patients suffer most from cognitive decline and hallucinations, while in others parkinsonism and autonomic failure are the predominant symptoms. Furthermore, motor symptoms are absent in more than 25% of DLB patients and, even when present, may be very mild in the beginning of the disease.[38] Due to the heterogeneity of the disease, the clinical diagnostic criteria have modest sensitivity and specificity. Even when criteria are correctly applied, the frequency of misdiagnosis is high due to substantial clinical overlap with other neurodegenerative diseases. As a consequence, more than 80% of DLB patients are initially diagnosed with other disorders, mainly AD or PD.[39] It is important to recognize that DLB and AD both exhibit cognitive decline and neuropsychiatric features and that in PD cognitive impairment and RBD are common. Further, given the frequent neuropsychiatric and mood disturbances, DLB can also be misdiagnosed as a psychiatric disease.

To improve the accuracy of the diagnostic criteria, the current iteration incorporated objective biomarkers - more specifically, PET and SPECT imaging and neurophysiologic measures - into the diagnostic criteria (see box 1).[36] Even though these techniques are sensitive, they are not always very specific for DLB, they are costly or involve radiation exposure. Most important, these biomarkers are indirect markers of the underlying disease mechanisms. This highlights the need to explore other (direct) biomarkers reflecting the disease-specific pathology of DLB.

Biomarkers for accurate and timely diagnosis of dementia with Lewy bodies

The challenge to diagnose DLB clinically, strengthens the importance of reliable and objective diagnostic tools and biomarkers. Characterization of direct biomarkers has been identified as a priority for DLB research and is a definite prerequisite for conducting clinical trials of emerging therapeutical targets. A biomarker is defined as a characteristic that is measured as an indicator of normal biological processes, pathogenic process, or responses to an exposure or intervention, including therapeutic interventions.[40, 41] A biomarker can support in identification of individuals at heightened risk of the disease, aid in diagnostic process, detect progression of the disease, as well as aid in the selection of patients for treatments or therapeutic trials and monitoring treatment response. In this thesis we mainly focused on the discovery of a diagnostic biomarker for DLB. A diagnostic biomarker is defined as a biomarker used to detect or confirm a disease or condition of interest or to identify individuals with a subtype of the disease.[40, 41]

The proximity of CSF to the brain parenchyma makes this biofluid the ideal source for biomarkers of neurodegenerative diseases. CSF potentially mirrors the biochemical alterations occurring in the brain. In addition, CSF is relatively easily accessible by lumbar puncture and CSF acquisition is safely, severe complications are rare and patients' acceptance is high.[42] AD has provided an example of the usefulness and clinical application of CSF biomarkers.[43] A typical CSF profile of decreased levels of amyloid-β 1-42 (Aβ42), combined with increased levels of total Tau protein (t-tau) and Tau phosphorylated at threonine 181 (p-tau) support the diagnosis of AD. The last few years, research on the discovery and development of CSF biomarkers for the diagnosis of DLB is emerging. Much of this effort is focused on the development of an α-synuclein biomarker, because of its role in the pathophysiology of DLB.

CSF α-synuclein

In DLB and other synucleinopathies CSF α-synuclein concentrations are typically lower compared with controls, whilst in AD and Creutzfeldt-Jakob disease, the α-synuclein concentrations are respectively slightly or extensively elevated in CSF. However, increased or unchanged CSF α-synuclein concentrations have also been reported in DLB (see [44-46] for review). The reasons for these conflicting results may be selection of patients with DLB/PD with differences in clinical characteristics in terms of age, disease stage, use of medication, as wells as the selection of different control groups ranging from healthy controls to neurological controls. Pre-analytical and analytical factors, such as the use of different immunoassays and antibodies, variation in pre-analytical processing, and blood contaminations from traumatic lumbar puncture - which have been shown to increase α-synuclein levels[44] - could also contribute to the large variation in absolute concentration of total α-synuclein and limit the comparability between studies. In addition, the large overlap of α-synuclein levels between groups hampers the clinical applicability of α-synuclein as a CSF biomarker for DLB and makes interpretation of the concentration for individual patients difficult. Moreover, current available assays for α-synuclein measure total amounts of the protein and not pathogenic specific isoforms. The development of robust and specific assays to quantify more disease-specific α-synuclein species, e.g. oligomeric α-synuclein and phosphorylated α-synuclein at Ser129 might resolve this issue.

CSF AD biomarkers

Understanding of the contribution of AD pathology to DLB is important. A major drawback of pathology studies, however, is that they reflect end-stage disease and it is questionable whether we can translate postmortem data to patients in the daily clinic. The study of CSF biomarkers enables to study AD-pathology *in vivo,* and before death. Previous CSF studies have shown that the established AD biomarkers - Aβ42, t-tau and p-tau – can also be abnormal in DLB patients (see [46, 47] for review). Most CSF AD biomarker studies in DLB, however, have included small cohorts from single centers, and only a few studies across the full spectrum of Lewy body diseases have been performed.[48, 49] A valid representation of the co-existence of AD pathology in early stages of the DLB is still missing.

Other isoforms of the amyloid-β (Aβ) peptide have also been proposed as CSF biomarkers for DLB. [50-55] In contrast with AD, reduced CSF levels of Aβ38, Aβ40 and Aβ42 in DLB have been reported in initial small studies.[50-55] However, CSF Aβ peptides have not yet been validated in large, well-characterized clinical cohorts and none of these studies addressed the issue of concomitant AD pathology in DLB. Therefore, the

influence of AD pathology on the proposed biomarkers CSF Aβ38 and CSF Aβ40 in DLB is unknown, whilst this is of particular importance for both diagnostic value and to elucidate the the putative role of altered amyloid-β metabolism in DLB.

Development of novel biomarkers for DLB

The discovery of novel biomarkers for dementias/neurological diseases is one of the most vibrant and important areas of research today. Although traditionally, biomarker discovery studies primary focused on the study of targeted individual proteins, technological advances enable researchers to simultaneous measure a large number of proteins present within a biological sample and to make data-driven, unbiased and hypothesis fee comparison of the proteome between healthy and diseases states. Mass spectrometry-based proteomics has emerged as a useful approach for unbiased candidate biomarker discovery. Proteomics studies not only could enhance the discovery of clinically relevant biomarkers, but would also elucidate molecular mechanisms underlying the disease. Despite significant improvements in the mass spectrometry technology, only few proteomic studies have yet been endeavored to identify novel CSF biomarkers for DLB.[56-58] Although these studies have presented a large number of proteins with differences in abundancies in DLB versus controls, there was no consensus between these three studies and none of the potential biomarker candidates has reached the phase of clinical implementation. Previous CSF proteomic studies in DLB have been limited by small sample sizes, often utilizing pooling strategies that limit the assessment of individual variability of potential markers, clinically heterogeneous patient cohorts and restricted validation of candidate biomarkers.[56-58] The limited reproducibility of previous studies can be overcome by modern proteomic pipelinesin order to enable high-throughput studies of well-defined samples and extensive validation steps.

GENERAL AIM AND OUTLINE OF THIS THESIS

The **general aim of this thesis** is to improve the (early) diagnosis of DLB by developing CSF biomarkers.

More specifically, we aim

1. to examine the diagnostic ability of pathogenic disease-associate forms of α-synuclein in CSF in patients with DLB;
2. investigate the impact of CSF AD biomarkers on diagnosis and prognosis in DLB;
3. identify and validate novel CSF biomarkers for DLB using an untargeted proteomic approach.

Outline of the thesis

The thesis is divided into two parts: existing CSF biomarkers and novel CSF biomarkers.

In the first part of this thesis we explore existing CSF biomarkers in DLB. In **chapter 2** we test the diagnostic ability of three CSF α-synuclein species, total α-synuclein, oligomeric α-synuclein and phosphorylated α-synuclein at Ser129, in DLB. In **chapter 3-5** we address the second aim of this thesis. In **chapter 3** we investigate the prevalence of CSF AD biomarkers across the full spectrum of Lewy body diseases in a large multicenter cohort and thereby providing a reliable and solid representation of the co-existence of AD pathology in early stages of the DLB. In **chapter 4** the predictive value of CSF Aβ42, t-tau and p-tau on longitudinal cognitive decline in DLB is evaluated. **Chapter 5** describes the analysis of amyloid-β peptide levels in CSF of DLB patients, AD patients and cognitively normal controls and examines whether these levels are influenced by the co-existence of AD pathology in DLB patients.

In the second part of this thesis we address the third aim and describe the discovery and validation of novel CSF biomarker candidates for DLB. **Chapter 6** covers the discovery of novel CSF biomarkers for DLB, using a high-throughput proteomic approach. Identified candidate biomarkers are thoroughly validated in three different validation steps. Additionally, we utilize machine learning to identify the biomarker panel best capable of classifying DLB patients. In **chapter 7** we further validate one of the most promising candidate biomarkers for DLB introduced in chapter 6, namely VGF, using two orthogonal analytical techniques.

Finally, in **chapter 8**, a summary is provided on the main findings of this thesis, followed by a general discussion and directions for future research to move the field forward.

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