



LUND UNIVERSITY

Biomarkers in Parkinson's disease and related disorders. Diagnostic value of biochemical markers and their relation to disease progression

Hall, Sara

2017

Document Version:
Other version

[Link to publication](#)

Citation for published version (APA):

Hall, S. (2017). *Biomarkers in Parkinson's disease and related disorders. Diagnostic value of biochemical markers and their relation to disease progression*. [Doctoral Thesis (compilation), Department of Clinical Sciences, Lund]. Lund University: Faculty of Medicine.

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Biomarkers in Parkinson's disease and related disorders

Biomarkers in Parkinson's disease and related disorders

Diagnostic value of biochemical markers and their
relation to disease progression

Sara Hall



LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended on January 13th 2017 at 13.00 in Belfragesalen, BMC, Lund,
Sweden

Faculty opponent
Professor Per Svenningsson

Organization LUND UNIVERSITY	Document name: Doctoral dissertation	
	Date of issue: January 13th 2017	
Author(s): Sara Hall	Sponsoring organization	
Title and subtitle: Biomarkers in Parkinson's disease and related disorders. Diagnostic value of biochemical markers and their relation to disease progression		
<p>Abstract</p> <p>Objectives: To identify diagnostic and prognostic biomarkers for Parkinson's disease (PD) and atypical parkinsonian disorders (APD) in blood and cerebrospinal fluid (CSF). To investigate longitudinal changes in CSF biomarkers in PD and to investigate the role of inflammatory biomarkers in CSF in PD and APD.</p> <p>Methods: We included patients and controls from the longitudinal, prospective Swedish BioFINDER study, but also from other clinical centers. We included patients with PD, PD with dementia (PDD), multiple system atrophy (MSA), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and for paper I also patients with Alzheimer's disease (AD) and dementia with Lewy bodies (DLB). Patients and controls underwent clinical assessment and testing regarding motor function and cognition. CSF and blood were analyzed using both newly developed and well established methods.</p> <p>Results:</p> <p>In paper I we assessed if a panel of CSF biomarkers could differentiate between common dementias and parkinsonian disorders. We found that levels of α-synuclein (α-syn) in CSF were decreased in the synucleinopathies (i.e. PD, PDD, DLB and MSA). The levels of NFL were increased in APD (i.e. MSA, PSP and CBD). Multivariate analysis revealed that a panel of five CSF biomarkers (α-syn, tau, P-tau, NFL and $A\beta_{42}$) could differentiate AD from PDD and DLB with a high diagnostic accuracy. NFL alone could differentiate PD from APD with a high diagnostic accuracy, multivariate analysis with the five CSF biomarkers did not improve the ability to distinguish between the different conditions.</p> <p>In paper II we investigated if levels of CSF biomarkers at baseline could predict cognitive decline and/or progression of motor symptoms after 2 years. We found that increased baseline levels of α-syn in the PD group correlated with cognitive decline and progression of motor symptoms. Low levels of $A\beta_{42}$ were associated with increased memory impairment. We also found strong correlations between α-syn and tau as well as P-tau in PD.</p> <p>In paper III we investigated the longitudinal changes in CSF biomarkers over a two year period. We found that α-syn, tau, P-tau, NFL and YKL-40 but not $A\beta_{42}$ increased over two years in PD but not in controls. However, α-syn and tau only increased in the PD group with disease duration > 5 years but remained stable early in the disease course. Furthermore, increase in P-tau and YKL-40 correlated disease progression.</p> <p>In paper IV we investigated if NFL in blood could differentiate between PD and APD. We found that NFL in blood correlated strongly with NFL in CSF. Furthermore we found that NFL in blood could differentiate between PD and APD with a high accuracy.</p> <p>In paper V we investigated the levels of inflammatory biomarkers in CSF of patients with PD and APD. We found that patients with PDD and MSA had higher levels of biomarkers of inflammation in CSF compared with PD and controls. The CSF levels of YKL-40 were decreased in PD compared with controls indicating astrocyte dysfunction. Inflammatory biomarkers correlated strongly with α-syn and markers of neuroaxonal injury (tau and NFL). In PD, higher levels of inflammatory biomarkers were associated with cognitive impairment and increased motor dysfunction.</p> <p>Conclusions: A panel of five CSF biomarkers can distinguish AD from DLB and PDD. Assessment of NFL in CSF as well as in blood can differentiate between PD and APD. The CSF levels of α-syn is decreased in the PD group as a whole but increase over time in those with long disease duration. Increased levels of α-syn at baseline within the PD group is associated with motor progression and cognitive decline and correlate with markers of neuroaxonal injury. We suggest that higher CSF levels of α-syn are associated with more intense neurodegeneration. The obtained data also suggests that inflammation is associated with a more aggressive disease in patients with parkinsonism.</p>		
Key words: Parkinson's disease. Atypical parkinsonism. Cerebrospinal fluid. Dementia.		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language: English
ISSN and key title 1652-8220		ISBN 978-91-7619-383-9
Recipient's notes	Number of pages	Price
	Security classification	

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature _____ Date _____

Biomarkers in Parkinson's disease and related disorders

Diagnostic value of biochemical markers and their relation to disease progression

Sara Hall



LUND
UNIVERSITY

Coverphoto by Olivier Le Queinec/Shutterstock

Copyright Sara Hall

Lund University
Faculty of Medicine
Doctoral Dissertation 2017:2

ISBN 978-91-7619-383-9

ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2016



To Henrik, Linn and Joel

Content

List of Publications	10
Abbreviations	11
Sammanfattning på svenska	13
Background and Introduction	15
Parkinson's disease	15
Atypical parkinsonism	22
The challenge of differential diagnosis in parkinsonism	24
Aims of the thesis	32
Material and Methods	35
The cohort	35
Ethical approval and patient consent	38
Blood and CSF samples	38
Paper I	38
Paper II	39
Paper III	40
Paper IV	41
Paper V	43
Results	45
Paper I	45
Paper II	49
Paper III	50
Paper IV	53
Paper V	57

Discussion	61
Improvement of the differential diagnosis	61
CSF biomarkers and neurodegeneration and disease progression	62
The association between tau and α -syn	63
Cognitive decline	64
Neuroinflammation in PD and APD	65
Neuroinflammation and mood in PD	66
Pre-analytic handling of CSF samples	66
Confounders	67
Limitations	68
Future directions	69
Conclusions	71
Acknowledgement	73
References	75
Permissions to print	91

List of Publications

This thesis is based on the following papers, which in the text are referred to by their Roman numerals.

- I. S. Hall*, A. Öhrfelt*, R. Constantinescu, U. Andreasson, Y. Surova, F. Bostrom, C. Nilsson, H. Widner, H. Decraemer, K. Nägga, L. Minthon, E. Londos, E. Vanmechelen, B. Holmberg, H. Zetterberg, K. Blennow and O. Hansson. "Accuracy of a Panel of 5 Cerebrospinal Fluid Biomarkers in the Differential Diagnosis of Patients with Dementia and/or Parkinsonian Disorders." *Archives of Neurology (JAMA Neurology)* 2012. Volume 69 (No. 11): 1445-52. *Equally contributed as first authors.
- II. S. Hall, Y. Surova, A. Öhrfelt, H. Zetterberg, D. Lindqvist and O. Hansson. "CSF Biomarkers and "Clinical Progression of Parkinson Disease." *Neurology* 84, no. 1 (2015): 57-63
- III. S. Hall, Y. Surova, A. Öhrfelt, the Swedish BioFINDER study, K. Blennow, H. Zetterberg and O. Hansson. "Longitudinal Measurements of Cerebrospinal Fluid Biomarkers in Parkinson's Disease." *Movement Disorders* 31, no. 6 (2016): 898-905.
- IV. O. Hansson, S. Janelidze, S. Hall, N. Magdalinou, A. Lees, U. Andreasson, N. Norgren, J Linder, L Forsgren, R Constantinescu, H. Zetterberg , K. Blennow, for the Swedish BioFINDER study. "Blood-based NfL: A Biomarker for Differential Diagnosis of Parkinsonian Disorders". *Neurology*, in press.
- V. S. Hall, S. Janelidze, Y. Surova, H. Widner, H. Zetterberg, O. Hansson. "Cerebrospinal fluid concentrations of inflammatory markers in Parkinson's disease and atypical parkinsonian disorders". *Manuscript*.

Reprints were made with the permission of the copyright owners.

Abbreviations

A β ₄₂	Amyloid beta 1-42
AD	Alzheimer's disease
APD	Atypical Parkinsonian Disorders
α -syn	α -synuclein
CBD	Corticobasal degeneration
CBS	Corticobasal syndrome
CNS	Central nervous system
DAT	Dopamine Transporter
LEDD	Levodopa Equivalent Daily Dose
MCI	Mild cognitive impairment
MRI	Magnetic Resonance Imaging
MSA	Multiple system atrophy
NfL	Neurofilament Light
PD	Parkinson's disease
PDD	Parkinson's disease with dementia
DLB	Dementia with Lewy bodies
PET	Positron Emission Tomography
PSP	Progressive supranuclear palsy
P-tau	tau phosphorylated at Thr181
SPECT	Single Photon Emission Computer Tomography

Sammanfattning på svenska

Parkinsons sjukdom (PD) är efter Alzheimers sjukdom (AD) den näst vanligaste sjukdomen där nervsystemet bryts ned, en s.k. neurodegenerativ sjukdom. PD förekommer hos ca 1% av befolkningen över 60 års ålder. Den är fortskridande och även om vi idag har mediciner för att behandla symptom finns ingen botande eller bromsande behandling.

Vid PD ansamlas proteinet α -synuclein (α -syn) i dopaminproducerande celler. Sjukdomsprocessen tros börja upp till 20 år innan patienten får motoriska symptom. Även inflammation i nervsystemet har kopplats till utveckling av PD.

Kliniskt utmärks PD av långsamma rörelser (bradykinesi), stelhet (rigiditet) och skakningar i vila. PD debuterar i allmänhet i ena sidan för att allteftersom sjukdomen fortskrider påverka båda sidor. Med tiden drabbas även många patienter av demens. Man räknar med att ca 30% av Parkinson patienter har demens (PDD) och att ytterligare 20-25% har en lindrig kognitiv svikt.

Det kan ibland vara svårt att ställa diagnosen PD, särskilt tidigt i förloppet då symptomen överlappar med s.k. atypisk parkinsonism (APD). APD utgörs av sjukdomarna multipel systematrofi (MSA), progressiv supranukleär pares (PSP) och kortikobasal degeneration (CBD). Dessa sjukdomar liknar ofta PD, men har ytterligare symptom, svarar dåligt på behandling och har sämre prognos. Det finns idag ingen undersökning eller prov som tidigt och säkert kan bidra till rätt diagnos. Vi har stora förhoppningar om kommande sjukdomsmodifierande behandlingar. Dessa behandlingar skulle dock behöva sättas in tidigt, innan sjukdomsprocessen fortskridit för långt.

För en tidig och säker diagnos behövs således förbättrade biomarkörer.

Man har under de senaste ca 20 åren studerat biomarkörer i ryggmärgsvätska/cerebrospinalvätska (CSF) extensivt. Dock finns ännu inte någon riktigt bra diagnostisk markör.

I detta forskningsprojekt har vi undersökt markörer i CSF och blod. Vårt mål har varit att identifiera markörer som möjliggör tidig och säker diagnos av PD och APD, identifiera markörer som kan förutse sjukdomsförloppet och undersöka hur dessa biomarkörer ändras över tid. Vi ville också undersöka inflammationens roll i PD och APD.

I artikel 1 fann vi att NfL (en markör för nervcells skada) i CSF var förhöjt i APD jämfört med PD och friska kontroller och att NfL kunde skilja mellan APD och PD med en hög diagnostisk säkerhet. Dock kan inte NfL bidra till att skilja mellan de olika APD. Vi fann också att α -syn är sänkt vid PD.

I artikel 2 undersökte vi hur biomarkörer i CSF vid det första besöket var associerat med förändring av kliniska symtom efter 2 års uppföljning vid PD. Vi fann att de PD patienter som hade högre nivåer av α -syn försämrades mer både avseende motoriska symptom och mental snabbhet över 2 år. Vi såg också att låga nivåer av $A\beta_{42}$ (ett protein som är lågt även vid AD) var associerat med större försämring av minnet efter 2 år.

I artikel 3 undersökte vi hur nivåerna av biomarkörer i CSF (α -syn, $A\beta_{42}$, tau, P-tau, NfL och YKL-40) ändras över 2 år vid PD och hos friska kontroller. Vi fann att alla markörer utom $A\beta_{42}$ ökade över 2 år vid PD men ej hos friska kontroller. Vi såg också att en större ökning av P-tau och YKL-40 hos PD patienter var associerat med klinisk försämring.

I artikel 4 undersökte vi NfL i blod hos patienter med PD, APD och friska kontroller. Vi fann att nivåer av NfL i blod korrelerade väl med nivåer av NfL i CSF. Vi fann också att NfL i blod kunde skilja mellan PD och APD med en diagnostisk säkerhet som var jämförbar med NfL i CSF.

I artikel 5 undersökte vi nivåer av inflammatoriska markörer i CSF hos patienter med PD, PDD, PSP, MSA och friska kontroller. Vi fann att patienter med PDD och MSA hade högre nivåer av inflammatoriska markörer än PD och kontroller. Ökade nivåer av inflammatoriska markörer var associerat med ökad motorisk och kognitiv påverkan vid PD.

Sammanfattningsvis fann vi att NfL i både blod och CSF är högre vid APD jämfört med PD och att denna markör kan bidra till att skilja mellan PD och APD-patienter. Detta kan bidra till en ökad diagnostisk säkerhet, även utanför högspecialiserade kliniker. Den kliniska undersökningen är dock fortsatt central. α -Syn är sänkt vid PD, sannolikt till följd av ansamling av α -syn i nervceller. Dock talar data även för att α -syn är associerat med neurodegeneration och högre nivåer inom PD gruppen är förenat med sämre prognos. Vidare bekräftar våra resultat rollen av inflammation i PD och vi föreslår att inflammation är associerat med ett mer aggressivt sjukdomsförlopp.

Background and Introduction

Parkinson's disease

Parkinson's disease (PD) was first described in 1817 by James Parkinson^{1, 2}. The disease was in 1872 described in further depth and given its name "Parkinson's disease" by Jean-Martin Charcot³ and is now recognized as the second most common neurodegenerative disease, second only to Alzheimer's disease. PD is rare before the age of 50 but the prevalence increases with age, affecting approximately 1% of the population over 60 years⁴⁻⁶. A Swedish study has recorded the highest incidence rates described, with >100/100.000 in the age group of 80 and older⁷. Some studies report that PD more commonly affects men than women; however, other studies report no differences⁴⁻⁶. In a Swedish study, the men – female ratio was 1.2:1⁷. More than causing suffering for the individual patient, PD also affects caregivers and society, with an estimated annual cost in Sweden of > 1.7 Billion SEK in 2009⁸. With an increasingly aging population PD can be expected to cause an increasing burden on society.

Pathophysiology of Parkinson's disease

Parkinson's disease is neuropathologically characterized by the inclusions of Lewy bodies and Lewy neurites containing α -synuclein (α -syn)⁹⁻¹². It is suggested that these α -syn containing inclusions lead to synaptic dysfunction, interfere with axonal transport and thus leads to neuronal damage of vulnerable, neuromelanin rich neurons in the dopaminergic substantia nigra pars compacta's (SNc) caudal and ventrolateral regions. The degeneration of the SN leads to further degeneration of the nigrostriatal system causing dopaminergic loss in the striatum causing the core motor features of PD¹¹. Neuropathological studies indicate a sequential spreading of the disease process starting in the medulla oblongata, spreading in a cranial direction and eventually affecting the cerebral cortex (the Braak staging system)⁹. It was subsequently revised to also include the anterior olfactory structures in stage 1 (Figure 1)¹³.

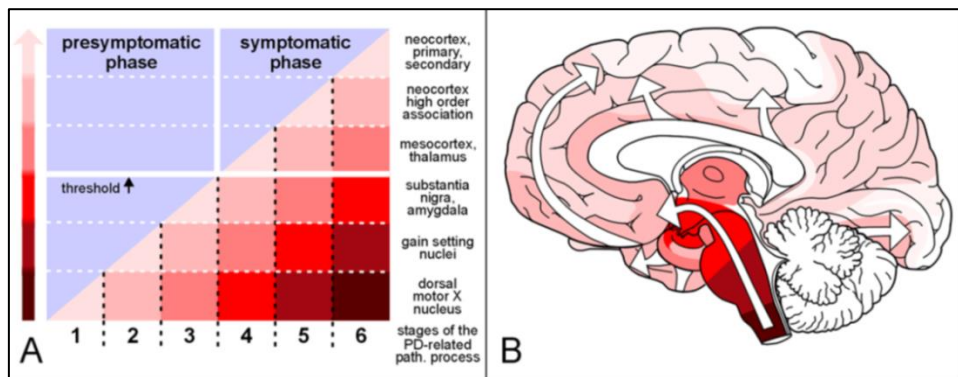


Figure 1A, B: PD presymptomatic and symptomatic phases. **A** The presymptomatic phase is marked by the appearance of Lewy neurites/bodies in the brains of asymptomatic persons. In the symptomatic phase, the individual neuropathological threshold is exceeded (black arrow). The increasing slope and intensity of the colored areas below the diagonal indicate the growing severity of the pathology in vulnerable brain regions (right). The severity of the pathology is indicated by darker degrees of shading in the colored arrow left. **B** Diagram showing the ascending pathological process (white arrows). The shading intensity of the colored areas corresponds to that in **A** (and in Fig. 4). **C** Composition of the human cerebral cortex. The allocortex (red) consists of the olfactory bulb, entorhinal region, and hippocampal formation. The extensive neocortex with its parietal, temporal, and occipital lobes consists of primary sensory fields (dark blue), first order sensory association areas (light blue), and the related high-order sensory association areas (orange). Similarly, the frontal neocortex consists of a primary motor field (dark green), premotor areas (olive), and prefrontal areas (yellow). Cell and Tissue Research, Stages in the development of Parkinson's disease-related pathology, Volume 318, 2004, pp 121–134, Heiko Braak, Estifanos Ghebremedhin, Udo Rüb, Hansjürgen Bratzke, Kelly Del Tredici. With permission of Springer.

Even though the Braak staging system has been criticized¹⁴ and another staging system has been suggested¹⁵, it is the main theory regarding the neuropathological progression in PD. Observations in transplanted patients has indicated to a process of neuronal transfer of α -syn that may propagate the disease process^{16, 17}. In neuropathological studies, Lewy bodies have been found in approximately 10% of asymptomatic patients older than 60 years. It has been suggested that this indicates preclinical PD¹⁸. This is in accordance with the hypothesis that the α -syn pathology starts up to 20 years before motor symptoms and at the time the patients notice motor symptoms, there is a 30% loss of dopaminergic neurons in the SN and 50-60% loss of their axonal terminals¹¹ (Figure 2). This is in concordance with clinical evidence of prodromal symptoms 10 years before diagnosis¹⁹.

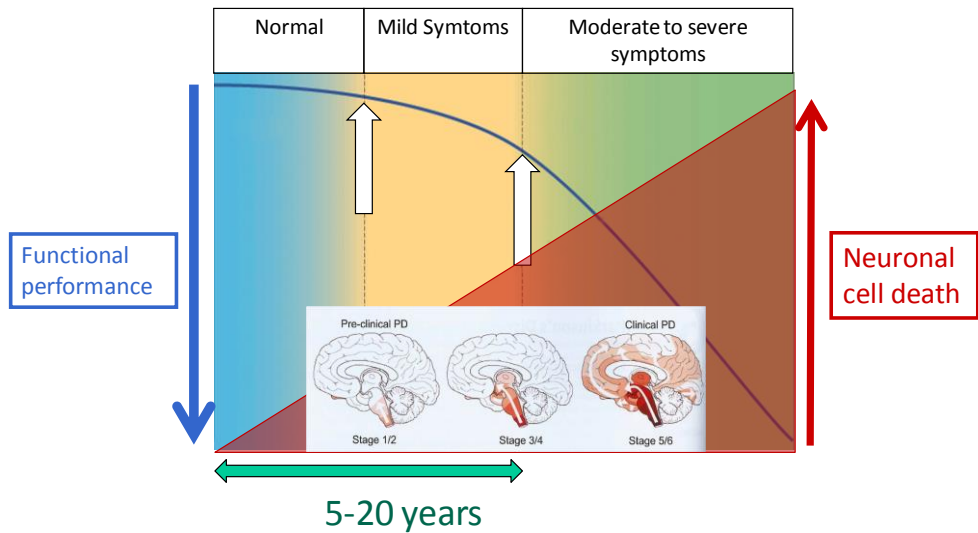


Figure 2. Image of relationship between neuronal cell death, Braak stage an clinical symptoms over time. With permission from Oskar Hansson

However, Lewy body pathology not only affects the central nervous system (CNS), including the olfactory bulb but also the spinal cord as well as multiple areas of the autonomic and peripheral nervous system, the parasympathetic system and the intramural enteric nervous system, the cardiac nervous system etc^{11, 20} explaining the multiple non motor symptoms affecting PD patient²¹. The neurodegenerative process causes a disruption in the normal, basal ganglia electrophysiological circuits including the motor, oculomotor, prefrontal and limbic circuit affecting both motor and cognitive function²². Furthermore, the neuropathology of cognitive symptoms are manifold and include degeneration of non-dopaminergic cells²³.

Neuroinflammation

Neuroinflammation has been implicated in the pathogenesis in PD and has been suggested to play an important role in neuronal death in dopaminergic cells in the substantia nigra^{24, 25}. Furthermore, dopaminergic neurons seem to be particularly vulnerable to inflammation²⁴. In the neuroinflammatory process, activated microglia has been particularly implicated. Microglia are the immunocompetent and phagocytic cells in the CNS²⁵. Microglia is sensitive to the local environment and when activated as a response to injury release substances such as cytokines, nitric oxide and glia-derived neurotrophic factor (GDNF). These substances may be pro- or anti-inflammatory, neurotrophic or neurotoxic leading to neurodegeneration²⁶⁻²⁸. Cytokines may also in turn activate microglia and thus aggravate the inflammatory cascade even further²⁷. Microglia are particularly

abundant in the substantia nigra where 12% of the cell proportion is microglia²⁹ PET studies have shown increased microglia in relevant structures of the brain in PD patients as well as APD³⁰⁻³⁴. However, it is still not clear whether activated microglia are detrimental or beneficial. It has been suggested that whether these are detrimental or beneficial may depend on disease stage²⁵, but there is evidence of resulting toxic effects contributing to neurodegeneration²⁸. It has also been suggested that aggregated α -syn contributes to the inflammatory response in microglia in PD²⁸ and possibly also MSA³⁵.

Cytokines also activate astrocytes as a response to injury³⁶. In PD, astrocytes seem to have a protective effect but may also be detrimental^{37,38}. Furthermore, one study show that levels of α -syn positive astrocytes correlate with cell death in PD suggesting that α -syn may impair astrocyte function³⁹.

Genetics in PD

The majority of all PD cases are idiopathic. However, about 20% of early onset PD and less than 3% late onset PD patients have a monogenetic etiology⁴⁰. The first PD causing mutation was discovered in 1997 in the α -syn gene (*SNCA*) 1997⁴¹. Since then, further genes linked to PD have been discovered with mutations with autosomal dominant inheritance (*SNCA*, *LRRK2*, *VPS35* and *CHCHD2*) or autosomal recessive inheritance (*PARK2*, *PINK1*, *DJ-1*, *ATP13A2*, *PLA2G6* and *FBX07*)^{40, 42}. However, the mutations in these genes have a variable penetrance. *SNCA* is highly penetrant whereas the mutations in *LRRK2* have a more varied penetrance with some highly penetrant mutations and others estimated to be as low as 24%^{43, 44}. Furthermore, there are common genetic risk variants present in the general population with a very low penetrance, but that through interactions with other factors, for example environmental factors, may contribute to PD⁴³. The low proportion of Parkinson caused by causative genes and the incomplete penetrance of risk genes become apparent in results from a longitudinal study based on the Swedish Twin registry where the concordance for PD were 11% for monozygotic twins and 4% for same-sexed dizygotic twins⁴⁵.

Mutations may yield distinct PD subtype phenotype (*SNCA*, *PARK2*, *PINK1* or *DJ-1*), parkinsonism with more variable features (*LRRK2*) or parkinsonism with additional atypical features (*ATP13A2*, *PLA2G6* and *FBX07*). Furthermore, there are mutations to genes more commonly linked to other neurological disorders that also may give rise to parkinsonism, for example the *MAPT* gene (microtubule-associated protein tau) causing frontotemporal dementia⁴². Genome-wide association studies (GWAS) have identified numerous risk alleles⁴³.

Clinical presentation in PD

Motor symptoms in PD

The core motor characteristics of PD are bradykinesia, rigidity, resting tremor and postural instability⁴⁶.

Resting tremor is in many patients an easily recognized symptom, presenting at a frequency of 4-6 Hz in the distal parts of the extremities, mainly the hands. Resting tremor on PD typically disappears in action as well as in sleep⁴⁶. In some patients the tremor can be difficult to distinguish from dystonic tremor or essential tremor. However, about 25% of patients don't have resting tremor at all⁴⁷.

Bradykinesia, slowness of movement, is another hallmark of PD. Bradykinesia may lead to difficulties in both walking as well as in fine motor skills that are important in daily living. Also, it is the cause of the classical presentation with hypomimia (loss of facial expression), and micrographia as well as decreased arm swing.

Rigidity, an increase in muscle tone, which in contrast to spasticity is not dependent on the speed of the movement, is the same in during whole range of motion and is not associated with increased muscle reflexes, is the third hallmark of PD. Rigidity is a lack of relaxation of the antagonistic muscle to a muscle actively being contracted in a motion. There is a continuum of rigidity towards dystonia where the paired muscles are contracted at the same time. Rigidity can also be accompanied by the cogwheel phenomena, which is rigidity and tremor simultaneously.

An asymmetric initial presentation is also a key feature whereas postural instability and freezing of gait are common features that typically present at a later stage⁴⁶.

A prodromal phase has been described. In a retrospective clinical case control study in a primary care setting, the earliest symptoms recorded that were overrepresented in the group that later developed PD were found 10 years ahead of diagnosis, supporting the hypothesis of the pathophysiological process starting years before the onset of motor symptoms¹⁹. Indeed, the prodromal phase has gained a lot of interest over the last few years and diagnostic criteria for prodromal PD were recently proposed⁴⁸.

Non-motor symptoms

Non-motor symptoms are less recognized than motor symptoms but have gained attention during the last decade⁴⁹. Non-motor symptoms are for many patients an important cause of decreased quality of life^{50, 51} and include psychiatric manifestations such as anxiety and depression; dysautonomia such as orthostatic

symptoms, urinary symptoms and constipation; as well as cognitive impairment, REM sleep behavior disorder (RBD), excessive daytime sleepiness, restless legs, pain and anosmia⁴⁹. Some non-motor symptoms can precede motor symptoms by several years, during the prodromal phase. Especially RBD and anosmia have therefore been suggested for screening to identify prodromal or very early PD^{52, 53}.

Parkinson's disease with dementia

Although considered a non-motor symptom, Parkinson's disease with dementia (PDD) merits its own paragraph. The dementia in PD differs from Alzheimer's disease (AD) in cognitive profile with more prominent visuospatial difficulties, and reduced attention and executive dysfunction. These differences between PD and AD are also reflected by differences in neuropathology²³. Memory may also be affected but the mild PDD patient can have a rather well preserved memory⁵⁴. This may lead to misdiagnosis of PDD as many of the test instruments commonly used to identify cognitive impairment focus on memory. Apathy, hallucinations, delusions, excessive daytime sleepiness and personality change are also common associated symptoms⁵⁵. The prevalence of dementia in PD is around 30%, and additional 20-25% are affected by mild cognitive impairment⁵⁶. The life time incidence of dementia in PD is estimated at around 75-80%^{56, 57}. Dementia typically does not occur until after about 10 years into the disease course, however, there is a large variability and 15-20% of PD patients have mild cognitive impairment (MCI) at the time of diagnosis⁵⁶. It is important to note that this aspect of MCI in the early course of the disease may improve after treatment with dopaminergic medication. MCI is a major risk factor for developing dementia, indeed in a recent study, all PD patients with incident MCI progressed to dementia in 5 years⁵⁸. However, pseudo-dementia factors such as lack of sleep, depression and under medication of parkinsonism need to be ruled out.

Diagnostic criteria

As the diagnosis for PD is clinical, diagnostic criteria are key components in the diagnosis. For PD there have been two rather similar and both widely accepted sets of criteria, the Queen Square brain bank criteria for Parkinson's disease⁴⁷ and the NINDS Diagnostic criteria for Parkinson's disease⁵⁹. For this study we have chosen the NINDS Diagnostic criteria (Table 1). There is an ongoing work to redefine the clinical diagnostic criteria for PD and a committee set up by the International Parkinson's disease and Movement Disorders Society (IPD-MDS) has recently introduced new criteria including non-motor symptoms in the definition as well as criteria for the prodromal phase^{48, 60}.

Table 1.

Diagnostic criteria for PD, adapted from according to Gelb et al, Arch Neurol 1999

Group A Features (clinical characteristics of PD)	
	Resting tremor
	Bradykinesia
	Rigidity
	Asymmetric onset
Group B features (suggestive of alternative diagnoses)	
	Prominent postural instability within the first 3
	Freezing phenomena within the first 3 years
	Hallucinations unrelated to medications within the first 3 years
	Supranuclear palsy or slowing of vertical saccades
	Severe, symptomatic dysautonomia unrelated to medication
	Documentation of condition known to produce parkinsonism and plausibly connected to the patient's symptoms
Criteria for Possible diagnosis of PD	
	At least 2 of the 4 features in group A; at least 1 of these is tremor or bradykinesia
and	None of the features in Group B are present OR Symptoms have been present for less than 3 years, and none of the features in Group B are present to date
and	Substantial and sustained response to levodopa or a dopamine agonist has been documented OR Patient has not had an adequate trial of levodopa or dopamine agonist
Criteria for Probable diagnosis of PD	
	At least 3 of the 4 features in Group A are present
and	None of the features in Group B are present (a symptom duration of at least 3 years is necessary to meet this requirement)
and	Substantial and sustained response to levodopa or a dopamine agonist has been documented.

There is also a set of diagnostic criteria of PDD⁵⁵. The diagnosis for PDD is based on the core features of a preexisting diagnosis of PD, dementia with insidious onset and slow progression that leads to a cognitive decline compared with premorbid function. The cognitive impairment should be present in more than one cognitive domain and must be severe enough to cause impairment in activities of daily living. The cognitive difficulties should also be present over a period of 6 months or more and be unresponsive to medication. There are also associated

cognitive and behavioral features. Cognitive features include different cognitive domains (attention, executive function, visuospatial function, memory and language) and behavioral features include apathy, personality and mood changes, hallucinations, delusions and excessive daytime sleepiness.

Atypical parkinsonism

Atypical parkinsonian disorders are rare, progressive neurodegenerative disorders. They are often, especially early on in the disease course difficult to distinguish from idiopathic PD as the different diagnoses share many features. However, they typically have some additional features, often termed “red flags”, such as early falls, symmetric onset, autonomic dysfunction, ophthalmoplegia, cerebellar dysfunction or pyramidal signs. Patients with atypical parkinsonism also generally have poor response to dopamine replacement therapies and the disease course is often more aggressive.

Multiple system atrophy (MSA)

MSA is a sporadic neurodegenerative disease that includes the degeneration of multiple neurological systems. This results in a heterogeneous phenotype that includes combinations of symptoms from two or more of the domains parkinsonism, cerebellar impairment, autonomic dysfunction and pyramidal tract signs. Many patients also suffer from dysphagia, stridor and dysarthria. Non-motor symptoms such as anxiety, depression, emotional incontinence, REM sleep behavior disorder and excessive daytime sleepiness can also occur. Although dementia is not a prominent feature, some patients also show some attention deficits and frontal dysfunction⁶¹. MSA is a rare disease, with an estimated mean incidence of 0.6-0.7 affected persons per 100,000 per year. The disease usually presents in the sixth decade of life⁶¹. MSA can be classified into two subtypes, depending on the dominant motor phenotype, parkinsonian MSA (MSA-P) or cerebellar MSA (MSA-C). MSA-P seem to be more prevalent in western countries whereas MSA-C is more common in Asia⁶².

MSA is neuropathologically characterized by glial cytoplasmic inclusions of α -synuclein, neuronal loss and axonal degeneration but also myelin degeneration and microglia activation. The glial cytoplasmic inclusions and the neurodegeneration that follows occur primarily in the striatonigral system, the olivopontocerebellar region, autonomic nuclei of the brainstem and the spinal cord⁶³. The prognosis is poor with a mean survival of 6-10 years from onset of symptoms⁶¹. The diagnosis

is based on clinical criteria⁶⁴ that over the years have been updated to also include imaging in additional features⁶⁵.

Progressive supranuclear palsy (PSP)

PSP is also a rare, sporadic, adult onset, progressive neurodegenerative disorder with a prevalence of 5.8-6,4 per 100,000⁶⁶. PSP was originally described by J.C Richardson, J.C Steele and J. Olszewski in 1963 when they described a progressive neurological syndrome characterized by supranuclear ophthalmoplegia, particularly downward gaze, pseudobulbar palsy, dysarthria, dystonic rigidity of the neck and upper trunk and dementia. They described eight patients who also presented with personality changes, axial rigidity and unsteady gait⁶⁷. This syndrome has subsequently become known as PSP-Richardson's syndrome (PSP-RS). The classic form of PSP, PSP-RS usually presents in the mid-60s and is characterized by postural instability, early falls, vertical supranuclear palsy, symmetric parkinsonism with bradykinesia and axial rigidity, pseudobulbar palsy and frontal disinhibition⁶⁸. However, PSP has over the years become known as a more heterogeneous disease with several phenotypes. These different phenotypes may over the disease course progress to PSP-RS. PSP with predominant parkinsonism (PSP-P) may, especially during the first years, be almost indistinguishable from PD and may also have a good levodopa response. However, early on, they develop dysphagia, dysarthria and eye symptoms even if these may be more subtle than in PSP-RS. PSP-P often has a more benign disease than PSP-RS. PSP with pure akinesia with gait freezing (PSP-PAGF) present with a prominent freezing of gait that does not respond to levodopa. They may also have akinesia but oculomotor signs and postural instability are late symptoms. There are several other phenotypes; PSP with corticobasal syndrome (PSP-CBS), PSP with predominant speech and/or language dysfunction (PSP-AOS and PSP-PNFA), PSP with predominant frontotemporal dysfunction (PSP-FTD), PSP with cerebellar ataxia (PSP-C) and PSP with primary lateral sclerosis (PSP-PLS)⁶⁹. PSP-RS typically has an aggressive disease course with a median survival of 5.6 years⁶⁸.

PSP is neuropathologically a tauopathy, with 4R (4 repeat isoform) tau deposits in glia cells, astrocytic tufts and neurofibrillary tangles particularly in the basal ganglia, diencephalon and the brainstem, but also in the cerebral cortex and white matter. Brain atrophy can be seen in the same areas. The distribution of tau pathology varies between the different phenotypes^{70, 71}

Even though a definite diagnosis is based on neuropathology, there are criteria for a clinical diagnosis. The diagnosis of probable PSP requires vertical supranuclear gaze palsy *and* postural instability with falls within the first year of disease onset.

Possible PSP however only requires vertical supranuclear palsy *or* slowing of vertical saccades and postural instability with falls within the first year⁷². There is also an ongoing work by the IPD-MDS committee on redefining the disease criteria.

Corticobasal degeneration (CBD)

CBD is yet another sporadic, adult onset neurodegenerative parkinsonian disease. CBD can present with different phenotypes with corticobasal syndrome (CBS) being the classical clinical presentation of CBD. CBS is characterized by asymmetric parkinsonism with limb rigidity and bradykinesia that is irresponsive to levodopa. Dystonia and myoclonus are also typical features. Higher cortical deficits such as apraxia, alien limb phenomenon and cortical sensory loss are also prominent features in CBS. Cognitive impairment is prevalent. However the clinical presentation is highly heterogeneous. Other phenotypes include Frontal behavioral-spatial syndrome, nonfluent/agrammatic variant of primary progressive aphasia, and progressive supranuclear palsy syndrome⁷³. CBD can thus present as Richardson syndrome and with that clinical presentation the pathology of PSP-RS is more likely but CBD-RS may occur making it a possible phenotype of CBD⁷⁴.

Neuropathologically CBD is yet another tauopathy with accumulation of 4R tau, however, compared to PSP, CBD has more cortical involvement. Also, CBD has astrocytic plaques compared to the tufted astrocytes in PSP⁷⁴.

The challenge of differential diagnosis in parkinsonism

As the different diseases and syndromes can be highly heterogeneous in themselves and are largely overlapping, the clinical diagnosis can in spite of existing clinical criteria be very difficult. Many patients, especially those with APD are diagnosed early on as PD, but as time passes, “red flag” symptoms develop and levodopa response is poor, the diagnosis may be changed, hopefully leading to a more correct diagnosis. Furthermore, to make the diagnosis of “probable” instead of “possible” PD, three years should have passed without symptoms related to “red flags” and with a preserved good levodopa response. Indeed, studies on the diagnostic accuracy of on parkinsonian syndromes have shown just how difficult the clinical diagnosis is. In a meta-analysis on the accuracy of the diagnosis of PD, studies where diagnoses have been made mainly by non-experts predictably show the lowest diagnostic accuracy with an accuracy of 73.8% (67.8-79.6). The accuracy improved if the diagnoses had been made by movement disorders specialist with an accuracy of 79.6% (46-95.1) at the initial

assessment and 83.9% (69.7-92.6) at follow-up. Using criteria can evidently help in the diagnosis of PD giving a pooled accuracy of 82.7% (62.6-93)⁷⁵. The diagnosis of atypical parkinsonian disorders is even more difficult. In a study on the diagnosis of parkinsonian syndromes by general neurologists PD was diagnosed with a sensitivity of 89.2% (79.1-95.6) but with a specificity of only 57.8% (42.2-72.3). The diagnosis of MSA and PSP had low sensitivity of 64.3% (35.1-87.2) and 52.9% (27.8-77.0) respectively but a high specificity of 99% (94.4-100) and 100% (96.2-100), respectively⁷⁶. The results again improve in a specialist movement disorder setting with a higher sensitivity of 88.2% and 84.2% in MSA and PSP respectively but with a specificity of 95.4 and 96.8% respectively⁷⁷. Thus there is a need for better diagnostic tools so that the patient can get a reliable diagnosis even if the patient isn't in a highly specialized clinic.

Are there tools that can help us today?

Imaging

Imaging of the dopaminergic system

Dopamine transporters (DAT) are responsible for the reuptake of dopamine released into the synaptic cleft and are expressed on the terminals of dopaminergic neurons in the striatonigral pathway. With the loss of dopaminergic neurons, there is also a loss of DAT. This can be investigated with single photon emission computer tomography (SPECT) or positron emission tomography (PET) imaging using ligands binding DAT, for example ¹²³I β-CIT (DopaScan™), ¹²³I FP-CIT (DatScan™) for SPECT or ¹⁸F FP-CIT for PET. DAT can in PD patients also be reduced as a consequence of reduced dopamine levels. The reduction of dopaminergic neurons can thus be overestimated with DAT imaging⁷⁸. In early PD, DAT imaging has showed a bilateral reduction in DAT binding in the putamen, being the most reduced in the posterior part contralateral to the most affected limb⁷⁹. In healthy elderly subjects there is a slight reduction in striatal DAT binding of 0.8% per year as measured by repeated ¹²³I β-CIT SPECT scans. However, the reduction is significantly higher in PD (11.2% per year)⁸⁰. DAT imaging can also be used to detect preclinical PD⁷⁸.

Studies have shown a high accuracy for DAT-SPECT imaging in differentiating patients with parkinsonian syndromes (PD and APD) from healthy subjects and essential tremor (ET) with a 87-98% sensitivity and 80-100% specificity^{81, 82}. Still, 4-15% of suspected early PD patients have normal DAT function when investigated with SPECT or PET. However these "subjects without evidence of dopamine deficit" (SWEDD) do not seem to progress significantly neither on imaging nor, but a few cases, clinically⁷⁸. Furthermore, DAT imaging is normal in drug induced parkinsonism and levodopa responsive dystonia. Also, imaging of

striatal DAT binding cannot distinguish between PD and atypical parkinsonian disorders (MSA, PSP and CBD) or DLB^{78, 81}. That being said, a longitudinal study showed that DAT binding reduction was faster in PD and PSP compared with MSA and healthy controls. Repeated DAT imaging could thus help improve the differential diagnosis⁸³. Regarding vascular parkinsonism results are conflicting, but DAT imaging may also show a reduction in DAT binding and even though a more diffuse uptake might indicate vascular PD⁸¹. DAT imaging can thus be an important tool in the diagnosis of PD and can be of use in identifying preclinical PD. However, a clinical assessment is still paramount.

Transport of dopamine into vesicles can be assessed with ¹¹C-DTBZ PET which targets vesicular monoaminergic transporter type2 (VMAT-2)⁸⁴. Binding of ¹¹C-DTBZ in the striatum has been found to correlate with motor dysfunction in PD. Uptake and storage of dopamine can be identified using the L-dopa precursor ¹⁸F-F-dopa PET⁸⁴. ¹⁸F-F-dopa correlates well with dopaminergic cell count and longitudinal studies using ¹⁸F-F-dopa have shown a decline in dopamine capacity at a considerably higher rate in PD compared with healthy elderly^{82, 85}. ¹⁸F-F-dopa PET studies have also shown correlations between decreased ¹⁸F-F-dopa binding and increased motor dysfunction in PD⁸⁶. However, there is no difference in diagnostic accuracy in between DAT imaging and ¹⁸F-F-dopa PET.

Magnetic resonance imaging

Traditional computer tomography and magnetic resonance imaging (MRI) can be useful to identify structural lesions that may cause parkinsonism but not to identify PD. In patients with parkinsonism however, MRI can be helpful in the differential diagnosis between PD and atypical parkinsonian disorders.

Studies using susceptibility weighted imaging (SWI) MRI sequences sensitive to iron have shown a hyperintense area in the dorsolateral part of the SN, the “swallow tail sign” in healthy controls whereas PD patients show a loss of this particular hyperintensity^{87, 88}. Loss of the dorsolateral nigral hyperintensity has also been shown to correlate with reduced DAT binding using ¹²³I-FP-CIT SPECT⁸⁹. However, the loss of the dorsolateral nigral hyperintensity is also seen in atypical parkinsonian disorders^{88, 89}. One small study using 7 Tesla MRI could show a loss of nigral hyperintensity in all patients with PD, MSA-P and PSP. However, this needs further validation⁹⁰. Substantia nigral hyperintensities may also be visualized by transcranial sonography (TCS) and studies suggest that TCS can differentiate between PD and controls and may better differentiate between PD and ADP than MRI. The investigation is however dependent on bone window and the expertise of the investigator⁹¹.

In MSA there is a considerable overlap between MSA-P and MSA-C. In MSA-P, putaminal atrophy with hyperintense putaminal rim and atrophy of the globus

pallidus are more pronounced; and in MSA-C infratentorial changes are more pronounced with atrophy of the middle cerebellar peduncle and pons, and dilatation of the fourth ventricle. The “hot cross bun sign” caused by T2 weighted hyperintensities in the pons has been shown to have a high positive predictive value of 97% but with a low sensitivity of 50% in comparison with other parkinsonian disorders and controls. However, the hot cross bun sign can also be seen in spinocerebellar ataxia⁹².

Patients with PSP typically show a midbrain atrophy with an enlargement of the third ventricle and preserved pons giving rise to the typical appearance of a humming bird “the hummingbird sign”. The width of the superior cerebellar peduncle has also been shown to be reduced in PSP. Whether these signs also are present early on in the disease course is unclear⁹².

In CBD patients typically have an asymmetric fronto-parietal atrophy and enlargement of the lateral ventricle⁹².

Diffusion tensor imaging (DTI) and diffusion kurtosis imaging (DKI), diffusion magnetic resonance imaging (dMRI) modalities, have shown white matter (WM) alterations in PD and clinical symptoms have correlated to diffusivity abnormalities⁸⁴ but the methods are still far from clinical use. Our group has shown that diffusion tensor tractography (DTT) and volumetrics could identify disease specific regional changes in white matter in foremost PSP and that these changes could help differentiate PSP vs. PD^{93, 94}.

FDG PET and rCBF –SPECT

¹⁸F-FDG PET reveals regional cerebral glucose metabolism whereas rCBF-SPECT reveals regional alterations in cerebral blood flow. In PD, ¹⁸F-FDG PET studies have revealed PD related metabolic patterns compared with healthy controls⁸⁴. However, the diagnostic accuracy when comparing PD from healthy controls is not high enough to use in clinical practice with 80% sensitivity and 78%⁹⁵. In contrast, FDG PET and rCBF-SPECT are used in clinical practice in atypical parkinsonian disorders and are incorporated in diagnostic criteria in MSA⁶⁵. FDG PET has in one study shown a 93% sensitivity and 83% specificity in distinguishing PD from APD⁹⁶ and another study found FDG PET to distinguish PD from MSA with 90% sensitivity and specificity⁹⁵.

In MSA, FDG PET studies have shown decreased metabolism in the putamen, brainstem and cerebellum and this is considered to be the most sensitive imaging test to distinguish MSA from other conditions⁹². Patients with PSP typically have reduced metabolism and rCBF in the frontal lobes as well as the striatum, thalamus and midbrain^{84, 92}. In CBD metabolism and rCBF can be reduced in the areas typically affected by atrophy, indeed asymmetry in metabolism and rCBF may be evident earlier than asymmetry on MRI^{84, 92}.

Cerebrospinal fluid biomarkers

α -Synuclein

α -Synuclein (α -syn) is a 140 amino acid presynaptic protein. The physiological role of α -syn is unclear but has been implicated in neurotransmitter release and vesicle transport⁹⁷. Under physiological conditions, α -syn exists mainly in a monomeric form⁹⁸ but tetrameric and multimeric forms have also been reported^{99, 100}. Oligomeric α -syn species are thought to be neurotoxic and have been implicated in the formation of Lewy bodies and Lewy neurites¹⁰¹. α -Syn is mainly found in the intracellular space, but can also be found in CSF and in blood, where it is abundant in erythrocytes. Due to its abundance in erythrocytes, it is important in CSF studies to not include samples with blood contamination¹⁰².

In CSF, α -syn has in many studies been found to be decreased in PD compared with healthy controls¹⁰²⁻¹¹⁰ whereas other studies have found no significant differences^{111, 112}. However, α -syn is also decreased on other synucleopathies i.e. DLB and MSA^{103, 105, 112, 113}. In contrast α -syn has been shown to be increased in both Alzheimer's disease¹⁰³ and Creutzfeldt-Jakob disease¹⁰⁷. One longitudinal study has shown that an increased level of α -syn within the PD group is associated with future cognitive decline¹¹⁴. In contrast, a study on early, untreated PD patients found lower α -syn to be associated with decreased cognitive performance, in particular on the executive-attention domain¹⁰⁸.

Not only has total α -syn but also oligomeric α -syn been investigated; levels of oligomeric α -syn are higher in PD and PDD compared with controls¹¹⁵⁻¹¹⁸, correlate with motor and cognitive dysfunction¹¹⁹ and increase further over a 2 year follow-up period¹²⁰.

Levels of phosphorylated α -syn in CSF have also been investigated. One study found increased levels of α -syn phosphorylated at serine 129 (pS129) in PD compared with controls in the discovery cohort but not in the validation cohort but decreased in both PSP and MSA in both cohorts. They also found a weak correlation between pS129 and disease severity as measured by UPDRS III¹²¹. pS129 has also been shown to increase over time^{120, 122}. We have not seen these differences in our cohort (data not shown).

Tau

The tau protein is present in high concentrations in areas with non-myelinated cortical axons and is important in stabilizing microtubule. Its hyperphosphorylated form, P-tau, causes tau to detach from the microtubule leading to microtubule destabilization and thus impaired axonal function. P-tau has an important role in synaptic plasticity but is also the basis for the formation of fibrillary tangles that are central in AD pathology¹²³. Tau in CSF thus reflects neurodegeneration and

axonal damage whereas CSF P-tau reflects the phosphorylated state of tau in the CNS.

In CSF, tau and tau phosphorylated at Thr 181 (P-tau) are markers commonly associated with AD, where studies have shown increased levels of both tau and P-tau compared with controls^{124, 125}. In PD however, studies have shown normal^{104, 113, 126, 127} or slightly decreased levels of a tau as well as P-tau in CSF^{105, 106, 108}. One study has shown that the ratio P-tau/tau, as well as P-tau/A β_{42} , correlates negatively with rate of change of UPDRS and rate of change in tau correlate positively with rate of change in UPDRS¹²⁸. Another study showed that increased P-tau and the ratio P-tau/A β_{42} at baseline predicted future cognitive decline on both memory and executive function¹²⁹. Interestingly, one study on early, untreated PD patients with MCI showed reduced tau and P-tau compared with controls¹⁰⁸. On the other hand, studies on PDD have shown normal or increased levels of tau and P-tau in PDD compared with controls and higher levels compared with patients with PD¹³⁰⁻¹³².

A β_{42}

CSF A β_{42} is a biomarker reflecting amyloid pathology. A β_{42} is like tau and P-tau considered a core biomarker for AD where studies have shown decreased levels of A β_{42} ^{124, 125, 133}. In PD, studies have shown normal^{104, 113, 130} or decreased levels of CSF A β_{42} in PD with and without MCI or PDD compared with controls^{105, 106, 108, 126, 127, 131, 132}. Low levels of A β_{42} in non-demented PD patients have been shown to be associated with future cognitive decline and an increased risk of developing dementia (HR 9.9%)^{126, 134, 135}. Furthermore, low A β_{42} has in non-demented PD patients been linked to worse result on phonetic fluency¹³¹ and memory impairment¹²⁷. Also, lower levels of A β_{42} has been linked to the PIGD phenotype¹³⁶

NfL

Neurofilament is an important component of the axonal cytoskeleton. Neurofilament consists of three subunits, neurofilament light (NfL), medium (NfM) and heavy (NfH)¹³⁷. Neurofilaments are unique to the CNS and can therefore serve as markers for neuroaxonal damage. NfL is the most abundant of the filaments and is also small and the most soluble, making it the neurofilament of choice¹³⁸. NfL has been found to be increased in CSF in several diseases and conditions where neuroaxonal damage occur, such as traumatic brain injury¹³⁹, stroke¹⁴⁰, amyotrophic lateral sclerosis^{141, 142} and frontotemporal dementia^{143, 144}. In this context, more importantly, NfL has been found to be increased in atypical parkinsonian disorders (MSA, PSP and CBD) compared with not only healthy controls but also compared with PD^{113, 145-148}. Indeed NfL has in two studies been shown to discriminate between APD and PD with a high degree of accuracy (92%

and 85% respectively^{113, 145}. A couple of studies show slightly increased levels of NfL in PD compared with controls^{113, 126} but others show unchanged levels^{145, 146, 148}.

Correlations between biomarkers

Tau has been found to correlate positively with P-tau¹²⁸. Furthermore one group has shown positive correlations between α -syn and tau as well as P-tau in PD and controls¹⁰⁶. These results are however contradicted by results from another group showing a negative correlation between tau and α -syn in PD^{104, 112}.

Inflammatory biomarkers

YKL-40, also known as chitinase-3-like-1, is a glycoprotein that is upregulated under inflammatory conditions in both peripheral tissue and the CNS¹⁴⁹⁻¹⁵¹. In the CNS, YKL-40 is expressed mainly by astrocytes and microglia and is a marker of glial activation^{152, 153}. Concentrations of YKL-40 in CSF have been found to be increased in AD¹⁵⁴. Fewer studies have been performed on parkinsonian syndromes showing normal or decreased levels in PD^{113, 155, 156}, but increased in APD^{113, 156}. The cytokine IL-6 is involved in the acute phase response. A couple of studies have shown increased levels of IL-6 in *de novo* PD patients^{157, 158} and one study showed an inverse relationship between IL-6 and motor symptoms as measured by UPDRS¹⁵⁸. Furthermore, in one study, IL-6 has been shown to be increased in PD patients with cognitive impairment¹⁵⁹. Our group did not see any significant differences in IL-6 levels in CSF between PD and PDD, however we found CSF CRP to be increased in PDD compared with non-demented PD patients and controls and IL-6 did correlate with cognitive dysfunction as measured by MMSE. We could also show a correlation between CRP and depression and fatigue in PD patients¹⁶⁰.

Blood biomarkers

Over the years, attempts to find diagnostic biomarkers for neurodegenerative diseases have been unsuccessful¹⁶¹⁻¹⁶³. However, during the last few years NfL in plasma and serum measured by ultrasensitive immunoassays have in a few studies shown great promise with increased levels in neurodegenerative disorders and CNS injury showing a high degree of correlation with levels in CSF^{141, 142, 164}. A very recent study has shown increased levels of plasma NfL in PSP compared with controls¹⁶⁵.

The need for biomarkers

As described above, the clinical diagnosis of PD can be difficult, largely because of overlapping symptoms, especially early on in the disease course. In spite of numerous studies, there are to date, no validated blood or CSF based diagnostic biomarkers for PD. CSF NfL has over the last few years emerged as a reliable biomarker in differentiating PD from APD and has become a useful tool in the clinical setting. However, NfL cannot distinguish PD from healthy controls or between the different APDs. DAT imaging can distinguish between healthy controls and disorders with dopamine deficiency but not distinguish between the different disorders. SWI MRI can contribute to the diagnosis of PD in a highly specialized setting, but is not available everywhere. Therefore, there is a need for better biomarkers. Preferably a biomarker should be reliable, with a high degree of sensitivity and specificity, available close to the patient, be non-invasive and reasonably priced.

In spite of numerous studies, there is to date no disease-modifying treatment available for PD. However, a few clinical trials are on the way. The pathological process is thought to start over a decade before the occurrence of motor symptoms and when the patient comes to a diagnosis, a large proportion of dopaminergic neurons are already lost. Disease-modifying treatments would therefore likely be most effective if initiated as early as possible, preferably even before the start of motor symptoms¹⁶⁶. There is thus an urgent need for biomarkers for an early and certain diagnosis. Furthermore, PD is a heterogeneous disease and reliable biomarkers are needed for stratifying clinical trials. Moreover, further CSF studies can contribute to our understanding of early pathophysiological mechanisms that may lead to new disease models and disease-modifying treatments.

From the perspective of patients and caregivers, a correct and early clinical diagnosis is highly beneficial, even in the absence of disease-modifying treatment. An early and certain diagnosis reduces insecurities, unnecessary investigations, allows for an early start of symptomatic treatment and medication errors may be avoided. Biomarkers might also be able to provide prognostic information in cases where patients and caregivers want that information.

Aims of the thesis

The overall aims of this thesis are:

- To identify biomarkers in CSF and blood for an early and accurate diagnosis.
- To identify prognostic biomarkers on disease progression and find better methods to predict if an individual with PD will develop cognitive impairment and/or more aggressive motor progression. Markers predicting disease progression would be valuable in the clinic and in clinical trials to select subjects most likely to benefit from novel treatments.
- Investigate the longitudinal change in CSF biomarkers. To understand the temporal changes are of great importance if CSF biomarkers are to be used in clinical trials or in clinical practice.
- To increase our understanding of the disease and pathophysiological mechanisms.

The specific aims were

Paper I

To investigate levels of CSF biomarkers tau, P-tau, $A\beta_{42}$, α -syn and NfL in the different diagnostic groups PD, PDD, DLB, AD, MSA, PSP, CBD and healthy controls and if a combination of biomarkers can differentiate between the different diagnostic groups.

Paper II

To investigate if levels of the CSF biomarkers tau, P-tau, $A\beta_{42}$, α -syn and NfL in PD could predict progression of motor symptoms or cognitive decline over a two year follow up.

Paper III

To investigate if the levels CSF biomarkers tau, P-tau, $A\beta_{42}$, α -syn, NfL and YKL-40 change over a two year follow up period in PD and in healthy controls; and if that change in biomarker levels would correlate with motor or cognitive symptoms.

Paper IV

To investigate if NfL in serum or plasma can differentiate between PD and APD with as high an accuracy as NfL in CSF. We also investigated correlations between NfL in plasma/serum and CSF.

Paper V

To investigate levels of biomarkers for inflammation in CSF in PD, PDD, MSA, PSP and healthy controls and to investigate if inflammation correlates with disease severity.

Material and Methods

The cohort

In all the papers of this thesis, patients from the cohort with parkinsonian symptoms in the prospective and longitudinal Swedish BioFINDER (www.biofinder.se) study were included (Figure 3). For paper I and IV, we also collaborated with other centers to include additional patients (please see the methods section for each paper).

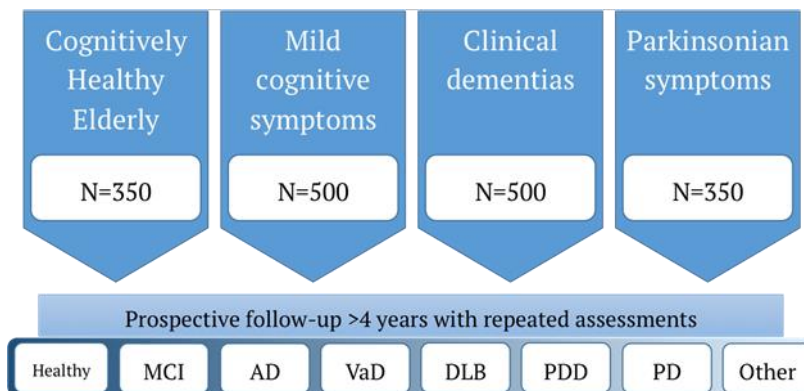


Figure 3 The BioFINDER Cohorts

As of December 2016, in the cohort with patients with parkinsonian symptoms who are recruited at the Neurology Clinic at Skåne University Hospital, we have included patients with PD ($n = 177$, of whom 58 were De Novo at baseline), PDD ($n = 28$), MSA ($n = 30$), PSP ($n = 26$), CBD ($n = 6$), dementia with Lewy Bodies (DLB) ($n = 14$), some with unclear diagnosis and five patients with Essential Tremor. We also include neurologically healthy controls ($n = 53$) at the same clinic, besides the larger cohort of healthy elderly individuals ($n=350$) recruited in Malmö. The study participants undergo assessment by a medical doctor with experience in movement disorders and a registered nurse using a large battery of rating scales (Table 2).

Table 2
Rating scales in the study

Rating Scale	Function	Reference
Motor tests		
Unified Parkinson Disease rating scale (UPDRS) I-IV	Measure of function	167
Hoehn & Yahr	Disease stage	168
Timed up and Go (TUG)	Ability of walking and turning.	169
Tandem gait	Balance	170
Cognitive tests		
Mini-Mental State Examination (MMSE)	Global test of cognitive function	171
Alzheimer's disease Assessment Scale (ADAS) item 1-3	Measures episodic memory recall	172
A Quick test of Cognitive speed (AQT),	Cognitive speed	173
1-minute Animal Fluency test	Verbal and executive function	174
1-minute Letter S Fluency test	Verbal and executive function	175
Clock drawing test	Visuospatial function	176
Questionnaires		
The International Quality Of Life Assessment (IQOLA), SF-12	Quality of life	177
Walk-12	Self assessed ability to walk.	178
SCOPA-AUT	Measures autonomic dysfunction	179
SCOPA-Sleep	Measure sleep and sleepiness	180
FACIT-FS	Measures fatigue	181
HADS	Measures anxiety and depression	182

A thorough medical history is taken and the patients undergo physical examination regarding symptoms of PD and APD according to criteria as well as exclusion criteria.^{55, 59, 64, 183-185} Levodopa equivalent daily dose is calculated¹⁸⁶. Controls undergo the same extensive testing, and individuals with overt signs of Parkinsonism or cognitive symptoms are not included in the study. Blood and CSF samples are obtained at baseline and biannually.

Study participants of the cohort with parkinsonian symptoms are followed for up to 10 years. At baseline and at the 2, 4, 6 and 10 year follow-up, the study participants undergo the examination as listed above. At the 1, 3, 5 and 8 year follow up patients are assessed with a shorter visit with a research nurse. Patients also undergo MRI at baseline and there after every other year (Table 3).

Table 3
Chart over follow-up routines in the study

	Baseline	1 year	2 years	3 years	4 years	5 years	6 years	8 years	10 years
Medical Doctor	✓		✓		✓		✓		✓
Registered nurse	✓	✓	✓	✓	✓	✓	✓	✓	✓
Medical history	✓		✓		✓		✓		✓
UPDRS I-IV	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hoehn & Yahr scale	✓		✓		✓		✓		✓
Timed Up and Go	✓		✓		✓		✓		✓
Tandem Gait	✓		✓		✓		✓		✓
MMSE	✓	✓	✓	✓	✓	✓	✓	✓	✓
ADAS item 1-3	✓		✓		✓		✓		✓
AQT	✓	✓	✓	✓	✓	✓	✓	✓	✓
Animal Fluency	✓		✓		✓		✓		✓
Letter-S Fluency	✓		✓		✓		✓		✓
Clock drawing test	✓		✓		✓		✓		✓
CSF	✓		✓		✓		✓		✓
Blood samples	✓		✓		✓		✓		✓
Questionnaires	✓		✓		✓		✓		✓
MRI	✓		✓		✓		✓		✓

Ethical approval and patient consent

All individuals gave informed written consent. The study procedure was approved by the local ethics committee at Lund University Sweden and conducted according to the Helsinki agreement

Blood and CSF samples

Plasma and CSF samples are collected with the patient non-fasting. CSF samples are collected in polypropylene tubes. Samples are centrifuged within 30 minutes at +4°C at 2000g for 10 minutes to remove cells and debris. Samples are stored in aliquots at -80°C pending biochemical analysis. The procedure and analysis of the CSF follow the Alzheimer's Association Flow Chart for CSF biomarkers¹³³. Samples are also sent to the local laboratory at Lund University Hospital for routine analyses in order to rule out previously undetected disease such as thyroid dysfunction or infection.

Paper I

Study participants and settings

This study was a collaboration between the Neurology Clinic at Skåne University hospital in Lund (from the Swedish BioFINDER cohort with parkinsonian symptoms), the Memory Clinic at Skåne University hospital in Malmö and Sahlgrenska University Hospital in Göteborg. In total we analyzed 453 CSF samples from patients with PD (n=90), AD (n=48), PDD (n=33), DLB (n=70), PSP (n=45), MSA (n=48), and CBD (n=12) and from healthy individuals serving as controls (n=107). All patients met diagnostic criteria^{55, 59, 64, 183-185, 187, 188}. Healthy controls had all undergone clinical and cognitive testing and individuals with objective parkinsonian or cognitive symptoms were not included in the study. Cognition was assessed with the MMSE and disease severity was assessed with Hoehn & Yahr.

CSF samples and hemoglobin test

CSF samples were collected and stored as described above. All samples were centrifuged within 30 min at +21°C (Sahlgrenska University Hospital) or at +4°C (Skåne University Hospital) at 2000g for 10 min. CSF hemoglobin levels were analyzed using a human hemoglobin enzyme-linked immunoassay kit (Bethyl Laboratories, Inc). Since our experiments using artificially blood-contaminated CSF showed that α -syn concentrations started to increase in CSF samples with hemoglobin concentrations above 1000 ng/L, only samples with hemoglobin levels below 1000 ng/L (405 of the 453 CSF samples) were included when studying CSF α -syn with non-parametric statistical methods.

NfL was analyzed using the NF-L enzyme-linked immunoassay (NF-light; UmanDiagnostics).

For simultaneous quantification of α -syn, A β ₁₄₂, tau and P-tau, a newly developed multiplex assay (Luminex) was used. Luminex data for A β ₁₄₂ and P-tau were normalized to ELISA concentrations by analyzing approximately 200 samples using both methods.

Statistical analysis

For basic statistics, the statistical analysis was performed using SPSS for Windows, version 18.0 (SPSS Inc). We then used a univariate general linear model, analysis of covariance using log-transformed biomarker levels correcting for confounding factors. We then performed a multivariate discriminant analysis (DA) using the orthogonal projections to latent structures (OPLS) algorithm implemented in commercial software (SIMCA P⁺ version 12; Umetrics). Receiver operating characteristic analysis was performed on the individual analytes as well as the results from the OPLS-DA using commercial software (GraphPad Prism, version 5; GraphPad Software).

Paper II

Study participants and settings

In this study, we included participants with PD from the cohort with parkinsonian symptoms from the Swedish BioFINDER study, who had a follow-up visit 2 years after baseline at the time when we started the analyses for paper II. These participants were also included in Paper I with baseline CSF. For comparisons of

baseline CSF levels we also included controls from the healthy elderly cohort of the Swedish BioFINDER study, also described in paper I. In total we included 42 non-demented PD patients and 69 controls.

CSF samples

CSF samples were obtained and handled as described for the cohort above. Levels of α -syn, $A\beta_{42}$, tau, p-tau, NfL and hemoglobin were determined as described in paper I. Samples with hemoglobin > 1000 ng/L were excluded when analyzing α -syn as done in paper I.

Statistical analysis

The statistical analysis was performed using SPSS for Windows, version 20.0. Due to the bimodal distribution of $A\beta_{42}$, this analysis was dichotomized using 550 ng/L as cut off¹²⁴. We used linear regressions to test associations between scores on clinical rating scales and CSF biomarkers, adjusting for age, gender, disease duration, and LEDD (levodopa equivalent daily dose), a calculated estimate of the amount of medication a patient is taking based on a levodopa effect comparison of various anti-parkinsonism medications¹⁸⁶. Before parametric analyses, non-normally distributed were normalized using log-transformation. However, in the case of change on clinical rating scale scores we used Blom's method for non-normally distributed data¹⁸⁹.

Paper III

Participants and settings

In this study, we included participants from the Swedish BioFINDER cohort with parkinsonian symptoms with lumbar puncture both at baseline and at the 2 year follow-up at the time we started the analysis of paper III. We included 63 patients with PD without dementia. 37 of the PD patients had short disease duration (≤ 5 years) and 26 had long disease duration (> 5 years). 11 of the patients with short disease duration were De Novo patients. We also included 21 neurologically healthy controls with repeated lumbar puncture at baseline and at the 2 year follow-up.

CSF samples

CSF samples were obtained as handled as described in papers I-II. Both baseline and follow-up samples were analyzed at the same time. CSF Ab42, tau, and P-tau were analyzed using Alz-Bio3 (Fujirebio, Ghent, Belgium), YKL-40 was analyzed using the Human Chitinase3-like 1 Quantikine ELISA Kit (R&D, Minneapolis, Minnesota), α -syn was analyzed using the Covance assay (Covance, Dedham, Massachusetts), NfL was analyzed using the NF-light assay (UmanDiagnostics, Umeå, Sweden), and hemoglobin was analyzed with an assay provided by Bethyl Lab, Inc. (Montgomery, Texas). In this study we did not perform our own experiments using artificially blood-contaminated CSF to investigate at what hemoglobin level α -syn concentrations started to increase. Therefore, we used hemoglobin < 200 ng/ml as cut-of point as established by a previous group¹⁰². Four baseline samples from PD patients and one baseline sample from the controls were subsequently excluded. For P-tau there were 16 missing in the PD group and eight missing in the control group. Baseline and follow-up CSF samples were always analyzed in the same batch. All analyses were performed using one batch of reagents.

Statistical analysis

The statistical analysis was performed using SPSS for Window version 22.0. To investigate changes in CSF biomarker levels over time we used a paired t test, or in the case of skewed data Wilcoxon signed-rank test. Linear regression was used to test for correlations between CSF biomarkers at baseline but also for correlations between changes in CSF biomarkers over 2 years and changes in clinical test scores as well as changes in other CSF biomarkers, correcting for age and LEDD.

Paper IV

Participants and settings

This study was a collaboration between four centers, forming three independent cohorts Skåne University Hospital, Lund with patients from the cohort with parkinsonian symptoms in the Swedish BioFINDER study (Cohort 1); National Hospital for Neurology and Neurosurgery, Queen Square, London (Cohort 2);

Sahlgrenska University Hospital, Göteborg and Umeå University, Umeå (Cohort 3 or Early disease cohort).

Cohort 1 (Lund cohort)

171 patients with PD, 30 with MSA, 19 with PSP, 5 with CBS and 53 neurologically healthy controls were included. All patients met diagnostic criteria^{59, 64, 183, 184}

Cohort 2 (London cohort)

20 patients with PD, 30 with MSA, 29 with PSP, 12 with CBS and 26 neurologically healthy controls were included. For PD the Queen Square Brain Bank Criteria was used⁴⁷, all other criteria were the same as in the Lund cohort

Cohort 3 (Early disease cohort)

Since the differential diagnosis between APD and PD is the most challenging during the first years of the disease, we included 53 patients with PD, 28 with MSA, 22 with PSP and 6 with CBS with early disease stage (disease duration ≤ 3 years). 26 neurologically healthy controls were also included. Patient in Cohort 3 met the same criteria as the London cohort.

Blood and CSF samples

Serum (London, Göteborg), plasma (Lund, Umeå), and CSF (London and Lund) were collected with the patients non-fasting, centrifuged and stored at -80°C within 30 minutes after collection.

CSF concentrations of NfL were measured with a sensitive sandwich method (NF-light® ELISA kit, UmanDiagnostics AB, Umeå, Sweden). In blood, NfL was measured using the monoclonal antibodies and calibrator from the NF-light assay, transferred onto the Simoa platform using a homebrew kit (Quanterix, Lexington, MA, USA)¹⁶⁴. Cohort 3 was analyzed separately from the Lund and London cohorts. We therefore included samples from the Lund cohort in this analysis to be able to normalize the NfL values between the cohorts.

In cohorts 1 and 2, the CSF levels of $A\beta_{42}$, and tau phosphorylated at Thr181 (P-tau) were analyzed using INNOTEST ELISA (Fujirebio Europe, Ghent, Belgium). CSF tau was analyzed with the EUROIMMUN ELISA (EUROIMMUN AG, Lübeck, Germany) in the Lund cohort and INNOTEST ELISA in the London cohort.

Magnetic Resonance Imaging

A subgroup of 102 study participants, including 39 controls, 89 PD, 7 PSP, 8 MSA and 2 CBS patients from the Lund cohort underwent MRI using a 3 T Siemens® system (Skyra). For assessment of WMLs standard T2 FLAIR images were used. Visual rating of WML on FLAIR images were performed according to the Fazekas scale¹⁹⁰.

Statistical analysis

SPSS (IBM, Armonk, NY, US) was used for statistical analysis. We excluded one outlier with plasma NfL value > 10 SD above the mean. Regression models were used to adjust for gender and age. The diagnostic accuracy of blood NfL was investigated using receiving operating characteristic (ROC) curve analysis.

Paper V

Study participants and settings

The study participants in this study were all from the parkinsonian symptoms cohort in the Swedish BioFINDER study. We included patients with PD (n = 131), PDD (n = 27), MSA (n = 24) and PSP (n = 14) with CSF samples. We also included 50 neurologically healthy controls with CSF samples.

CSF samples

The collection and handling of CSF samples are described for the cohort above. CRP, SAA, IL-6, IL-8 and MCP-1 were analyzed using V-Plex Custom Human Biomarkers kit (Meso Scale Discovery, Rockville, MD, USA). YKL-40 concentrations were measured by solid phase sandwich ELISA according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN, USA). α -syn was analyzed using an Alpha-synuclein ELISA kit (Meso Scale Discovery, Rockville, MD, USA). The analyses for tau, P-tau A β_{42} , Hb and NfL are described in paper IV (Hansson et al 2016). For α -syn only samples with hemoglobin < 200 ng/L were used.

Statistical analysis

The statistical analysis was performed using SPSS for Window version 22.0. Comparisons between CSF inflammatory biomarkers in the different diagnostic groups were made with a univariate linear model correcting for age. Correlations between levels of CSF biomarkers and correlations between CSF biomarkers and clinical test scores were analyzed with a linear regression correcting for age. Non-normally distributed data was log-transformed before parametric analyses.

Results

Paper I

In this study, we investigated levels of five different CSF biomarkers (α -syn, tau, P-tau, $A\beta_{42}$ and NfL) across the diagnostic spectrum of common dementias, parkinsonian syndromes and healthy controls. We then investigated if a panel of these five CSF biomarkers could help differentiating between the disorders.

CSF biomarkers in the different diagnostic groups

α -synuclein

We found that α -syn correlated with age in controls, PD and MSA ($R_s \geq 0.2$, $p \leq 0.05$). α -syn was decreased in PD and MSA compared with controls and patients with PSP ($p < 0.05$). The patients with PDD and DLB were significantly older than controls. After age matching the groups, α -syn was also decreased in PDD and DLB compared with controls. In AD on the other hand, α -syn was significantly increased compared with all other groups ($p < 0.05$, Figure 4). These differences between the groups withstood correcting for age, gender and hemoglobin levels.

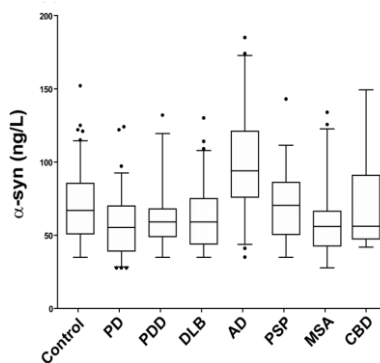


Figure 4. Boxplots of α -syn in the different diagnostic groups. The lower, upper and the middle lines of boxes correspond to 25th, 75th percentile and median, respectively. The whiskers at the top and bottom extend from the 95th and 5th percentile, respectively.

Traditional AD biomarkers

We found that patients with DLB had decreased $A\beta_{42}$ ($p < 0.001$) compared with controls and patients with PD. However, in AD, levels of $A\beta_{42}$ were even further decreased. Tau as well as P-tau was as expected increased in AD compared with controls, PD and PDD ($p < 0.001$). These results withstood correcting for age, gender and hemoglobin levels. In PD, tau and P-tau was decreased compared with controls ($p < 0.05$). P-tau was also decreased in PSP and MSA ($p < 0.05$). These results however did not withstand correcting for age, gender and hemoglobin levels.

NfL

NfL correlated with age in controls, PD and DLB ($R_s \geq 0.4$, $p \leq 0.01$). NfL also correlated with disease severity as measured by Hoehn & Yahr in PD and PSP ($R_s \geq 0.33$, $p < 0.01$) but not disease duration. In AD, NfL correlated with worse results on MMSE ($R_s = -0.035$, $p = 0.02$).

NfL was increased in all patients with atypical parkinsonism (i.e. MSA, PSP and CBD) as well as DLB compared with PD and controls ($p < 0.001$). Furthermore, NfL was increased in PDD compared with PD ($p < 0.01$). However, when correcting for age, gender and hemoglobin levels the significant difference between PD and PDD was lost. When correcting for age, gender and hemoglobin, controls had lower levels compared with all other groups ($p < 0.05$). After additional adjustment for Hoehn & Yahr score we still found a significant difference between APD compared with PD and PDD ($p < 0.05$, Figure 5).

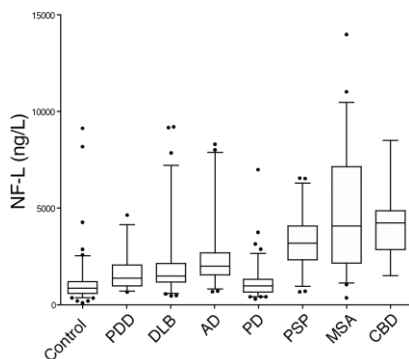


Figure 5. Boxplots of NfL in the different diagnostic groups. The lower, upper and the middle lines of boxes correspond to 25th, 75th percentile and median, respectively. The whiskers at the top and bottom extend from the 95th and 5th percentile, respectively.

Multivariate discriminant analysis (OPLS-DA)

When analyzing all five CSF biomarkers simultaneously we found that in the groups with predominantly dementia, a combination of the five biomarkers could differentiate AD from DLB and PDD with a sensitivity of 90% (95% CI, 83%-95%), a specificity of 81% (95% CI, 67%-91%) and an area under the curve (AUC) = 0.90 (95% CI, 85%-96%, Figure 6).

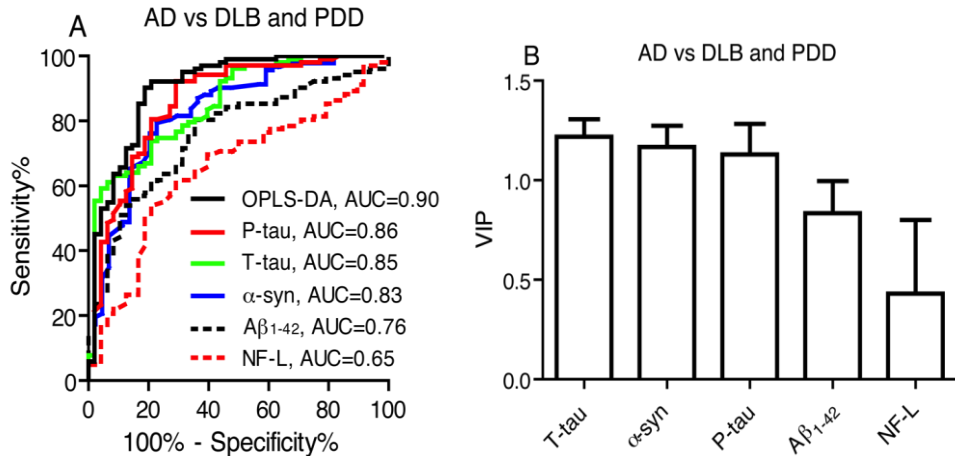


Figure 6.

A) Multivariate discriminant analysis (DA) was performed using the orthogonal projections to latent structures (OPLS) algorithm. Receiver operating characteristic curves were calculated for both OPLS-DA and for each analyte, including α -synuclein (α -syn), β -amyloid1-42 ($A\beta_{1-42}$), total tau (T-tau) phosphorylated tau (P-tau), and neurofilament light chain (NF-L). The areas under the curves (AUC) are given. B) Patients with AD were compared to patients with DLB and PDD. Corresponding variable importance in projection (VIP) plots illustrate the relative contributions of the analytes to the separation between the patient groups. Error bars represent the 95% confidence interval

Furthermore, in the groups with predominantly parkinsonism, the five different CSF biomarkers could together separate PD from APD with a sensitivity of 85% (95% CI, 76%-91%), a specificity of 92% (95% CI, 85%-97%) and AUC = 0.93 (95% CI, 89%-97%). In the multivariate analysis, NfL contributed the most to the model. Indeed, NfL alone could separate APD from PD a similar diagnostic accuracy to that of the multivariate model (Figure 7).

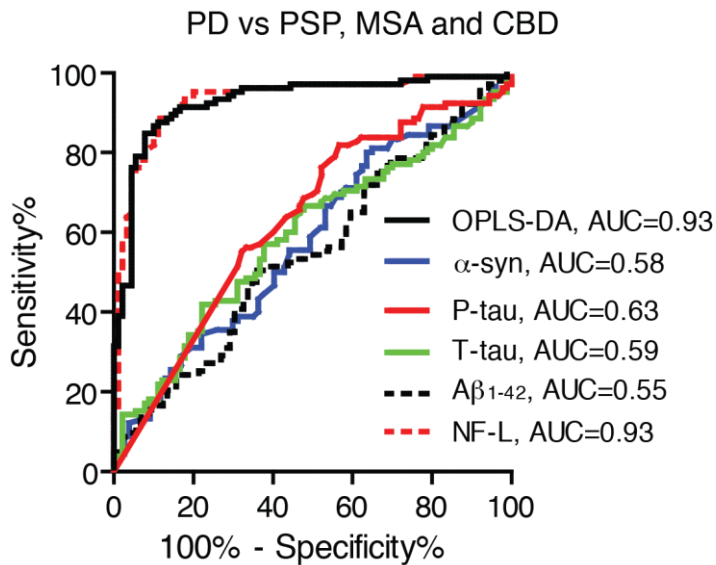


Figure 7. Multivariate discriminant analysis (DA) was performed using the orthogonal projections to latent structures (OPLS) algorithm. Receiver operating characteristic curves were calculated for both OPLS-DA and for each analyte, including α -synuclein (α -syn), β -amyloid1-42 (A β 1-42), total tau (T-tau) phosphorylated tau (P-tau), and neurofilament light chain (NF-L). The areas under the curves (AUC) are given comparing patients with PD to patients with atypical parkinsonism, i.e. PSP, CBD and MSA.

Paper II

In this study, we investigated if the CSF biomarkers assessed at baseline in paper I could predict cognitive decline and motor progression in PD.

Correlations between the different CSF biomarkers

There were extensive correlations between the different biomarkers at baseline. In both PD and controls α -syn correlated with tau ($R_s \geq 0.508$, $p \leq 0.001$), P-tau ($R_s \geq 0.597$, $p < 0.001$) and NFL ($R_s \geq 0.380$, $p < 0.020$) (Figure 8). Tau also correlated with P-tau in both groups ($R_s \geq 0.620$, $p < 0.001$). In the PD-group but not the control group, NfL correlated with lower levels of $A\beta_{42}$ ($R_s = -0.308$, $p = 0.050$).

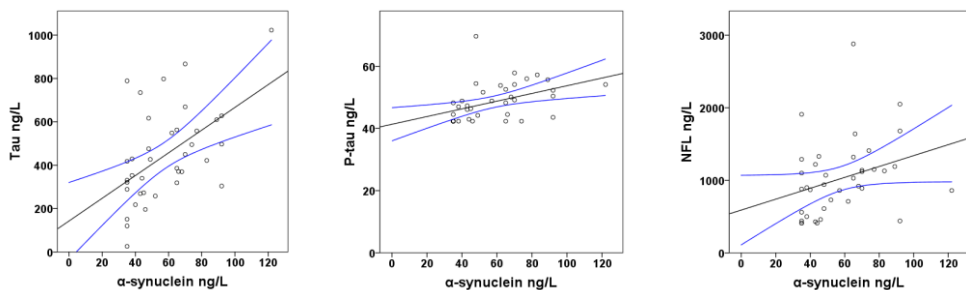


Figure 8. Correlations between levels of CSF biomarkers in PD. Black lines are the linear regressions, blue lines 95% confidence interval.

Correlations between CSF biomarkers at baseline and cognitive decline

Higher baseline levels of α -syn in the PD group correlated with deterioration of cognitive speed (AQT) over the 2 year follow up ($\beta = 0.423$, $p = 0.018$) (Figure 9). Furthermore, PD patients with baseline $A\beta_{42} < 550$ ng/L deteriorated more on delayed memory recall (ADAS item 3) compared with PD patients with normal levels of $A\beta_{42}$ ($F = 5.834$, $p = 0.022$), all correcting for age, gender, disease duration and LEDD.

Correlations between CSF biomarkers at baseline and motor progression

Higher levels of α -syn at baseline in the PD group correlated with increased motor dysfunction over the 2 year follow up period as measured by an increase in Hoehn

Yahr score ($\beta = 0,394$, $p = 0.043$), an increased UPDRS III score ($\beta = 0.449$, $p = 0.013$) (Figure 9) and prolonged TUG ($\beta = 0,406$ $p = 0.023$). Higher baseline of P-tau also correlated with increased motor dysfunction in PD as measured by an increase in Hoehn Yahr score ($\beta = 0,366$, $p = 0.038$) and an increase in UPDRS III score ($\beta = 0,350$, $p = 0.045$, all correcting for age, gender, disease duration and LEDD (Figure 9).

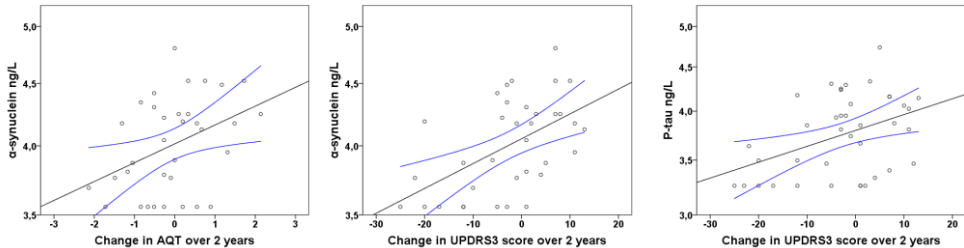


Figure 9. Correlations between CSF biomarkers at baseline and change in clinical test scores over 2 year. Black lines are the linear regressions, blue lines 95% confidence interval.

Paper III

In this study we investigated the temporal changes of the CSF biomarkers α -syn, $A\beta_{42}$, tau, P-tau, NfL and YKL-40 in PD and healthy controls. We also investigated if changes in levels of CSF biomarkers correlated with clinical progression.

Demographics and CSF biomarkers at baseline

In this study, we found no significant differences in CSF biomarker levels at baseline between the groups. In both PD and controls age correlated positively with NfL ($R_s \geq 0.517$, $p \leq 0.002$), and YKL-40 ($R_s \geq 0.525$, $p \leq 0.014$). In the PD group age correlated positively with α -syn ($R_s = 0.286$, $p = 0.028$), tau ($R_s = 0.394$, $p = 0.001$) and negatively with $A\beta_{42}$ ($R_s = -0.263$, $p = 0.037$).

Change over 2 years in CSF biomarker levels

In the control group there were no significant changes in CSF biomarkers over time. In the PD group, tau ($t_{62} = -2.570$, $p = 0.013$), P-tau ($t_{46} = -2.458$, $p = 0.018$), α -syn ($t_{58} = -2.350$, $p = 0.022$), NfL ($z = -3.769$, $p < 0.001$), and YKL-40 ($t_{62} = -3.682$, $p < 0.001$) increased over the 2 year follow-up (Figure 10).

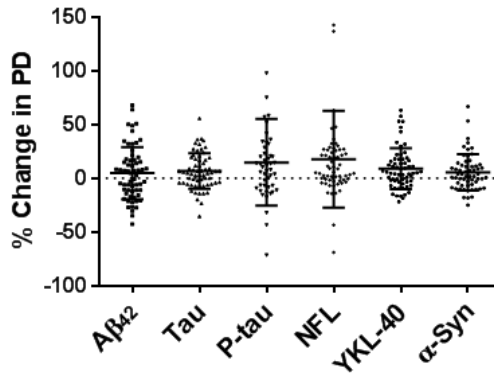


Figure 10. Scatter dot plot over percentual change i CSF levels in the different CSF biomarkers over 2 years. Lines represent means and standard deviations. One data point for P-tau (at 200%) and one data point for NfL (at 276%) are outside the axis limit.

In the PD group with short disease duration (disease duration ≤ 5 years, $n = 37$), CSF levels of NfL and YKL-40 increased over 2 years ($z = -3.079$, $p = 0.002$ and $t_{36} = -2.675$, $p = 0.011$) but not α -syn or tau. However, in PD with long disease duration (disease duration > 5 years, $n = 26$) there were significantly increased levels if α -syn ($z = -2.192$, $p = 0.028$), tau ($z = -2.437$, $p = 0.015$), NfL ($z = -2.210$, $p = 0.027$) and YKL-40 ($z = -2.222$, $p = 0.026$) over 2 years (Figure 11). Similar results were seen when separating the PD group by unilateral (i.e. Hoehn & Yahr score < 2) or bilateral (i.e. Hoehn & Yahr score ≥ 2) disease.

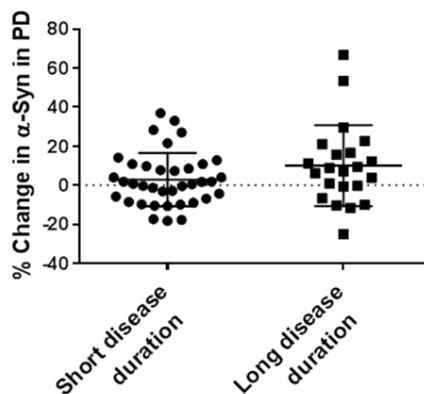


Figure 11. Scatter dot plots over percentual change in CSF levels of α -syn in PD with short and long disease duration. Lines display means and standard deviations.

Correlations between changes in CSF biomarker levels over 2 years in PD

There were extensive correlations between changes in CSF biomarkers over 2 years (Table 4). These correlations withstood correcting for age and LED.

Table 4

Correlations between changes in CSF biomarkers over 2 years in PD.

	A β ₄₂	Tau	P-tau	α -syn	NFL	YKL-40
A β ₄₂			p=0.004 r=0.408			
Tau			p<0.001 r=0.487	p<0.001 r=0.588	p=0.003 r=0.371	p=0.002 r=0.382
P-tau	p=0.004 r=0.408	p<0.001 r=0.487		p=0.005 r=0.424		p=0.008 r=0.381
α -syn		p<0.001 r=0.588	p=0.005 r=0.424			p<0.001 r=0.521
NFL		p=0.003 r=0.371				
YKL-40		p=0.002 r=0.382	p=0.008 r=0.381	p<0.001 r=0.521		

Correlations between changes in CSF biomarker levels and changes in clinical symptoms over 2 years in PD

An increase in P-tau over 2 years in the PD group as a whole correlated with deterioration in motor function as measured by UPDRS III ($\beta = 0.292$, $p = 0.048$) and in executive cognitive function as measured by Letter S fluency ($\beta = -0.440$, $p = 0.002$). An increase in YKL-40 correlated with worsening on Letter S fluency ($\beta = -0.276$, $p = 0.032$), all correcting for age and LEED (Figure 12).

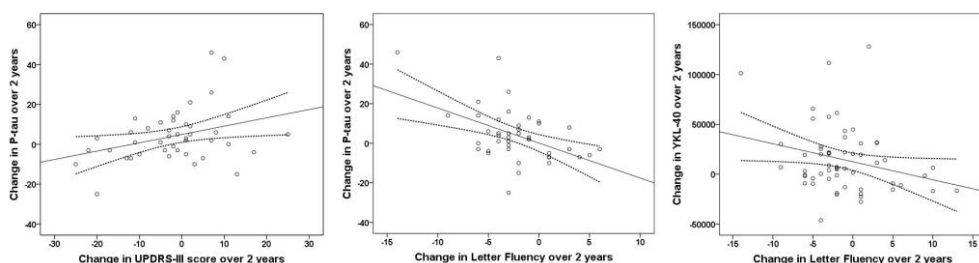


Figure 12. Correlation between changes in CSF biomarker levels over 2 years and changes in clinical test scores over 2 years in PD. Solid lines are linear regressions and dotted lines are 95% confidence intervals.

Paper IV

In this study we collaborated with three other centers to be able to if NfL in blood correlated with NfL in CSF and to assess if NfL in blood could differentiate between PD and APD in independent cohorts.

Demographics

In both the Lund and the London cohorts, we found that blood levels of NfL correlated with age in the cohort as a whole ($R_s \geq 0.290$, $p \leq 0.001$), controls ($R_s \geq 0.411$, $p \leq 0.037$) and patients with PD ($R_s \geq 0.483$, $p \leq 0.031$). In the Lund cohort women had higher blood NfL levels compared with men in the whole cohort ($p = 0.041$) and in the PD group ($p = 0.040$). In the London cohort women had higher levels of NfL in blood compared with men in the APD groups ($p = 0.048$).

Correlations between levels of NfL in CSF and Blood

The Lund Cohort

Blood NfL correlated with CSF NfL in the cohort as a whole ($\beta = 0.697$, $p < 0.001$) (Figure 13) as well as in each individual group; controls ($\beta = 0.344$, $p = 0.033$), PD ($\beta = 0.481$, $p < 0.001$) and APD (i.e. MSA, PSP and CBS) ($\beta = 0.523$, $p < 0.001$) all correcting for age and gender.

The London Cohort

Similar results were seen in the London cohort with a strong correlation between blood NfL with CSF NfL in the cohort as a whole ($\beta = 0.796$, $p < 0.001$) (Figure 13) as well as in controls ($\beta = 0.449$, $p = 0.022$) and APD (i.e. MSA, PSP and CBS) ($\beta = 0.677$, $p < 0.001$), all correcting for age and gender. The number of PD patients with CSF analysis was too low ($n = 5$) to allow for statistical analysis.

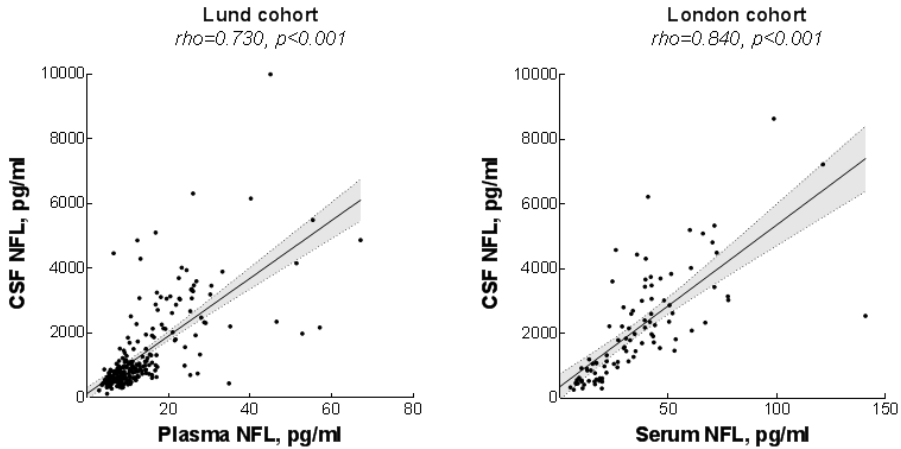


Figure 13. Correlations between NfL in blood and CSF in the Lund cohort and in the London cohort.

Blood NfL levels in the different diagnostic groups

The Lund cohort

The levels of NfL in blood were significantly higher in MSA, PSP and CBS compared with controls as well as PD (all $p < 0.001$), adjusted for age and gender (Figure 14). Also adjusting for disease duration did not affect the results.

The London cohort

Similar results were seen in the London cohort with significantly higher levels of NfL in blood in MSA, PSP and CBS compared with controls and PD (all $p < 0.001$), all adjusted for age and gender. Furthermore, in the London cohort, blood NfL was higher in PD compared with controls ($p = 0.011$) but still far below the levels of the APD patients, adjusted for age and gender (Figure 14). Also adjusting for disease duration did not affect the results.

The early disease cohort

In the early disease cohort with patients with disease duration ≤ 3 years blood NfL was significantly higher in MSA, PSP and CBD compared with PD (all $p < 0.001$), all adjusted for age and gender (Figure 14).

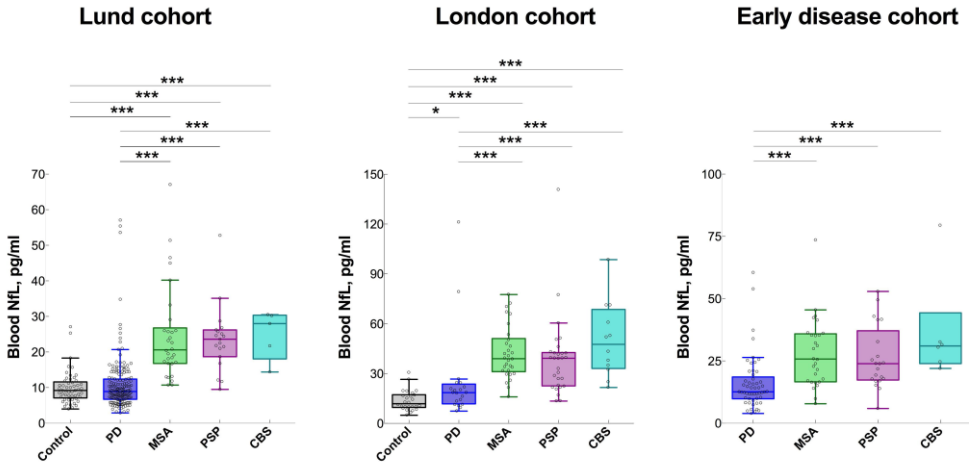


Figure 14. Scatter dots and box plots of blood NfL in the different diagnostic groups in the three different cohorts. Values are from a univariate linear model correcting for age and gender. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. One case with PSP in the early disease cohort had NfL concentration outside the axis limit but is included in the statistical analysis.

Diagnostic accuracy of blood NfL

The Lund Cohort

Blood NfL could distinguish patients with PD from patients with APD with a high diagnostic accuracy (82% sensitivity and 91% specificity, AUC = 0.91, 95% CI 0.87-0.95). Similar results were seen when analyzing the different APD groups (MSA, PSP and CBD) separately with > 80% sensitivity and > 80% specificity, AUC > 0.91. The diagnostic accuracy for NfL in blood to discriminate between PD and APD was comparable to that of NfL in CSF (92% sensitivity, 93% specificity and AUC = 0.96, 95% CI 0.92-0.99).

The London Cohort

Similarly, Blood NfL could in the London cohort distinguish patients with PD from patients with APD (80% sensitivity and 90% specificity, AUC = 0.85, 95% CI 0.72-0.98). Similar results were seen when analyzing the different APD groups (MSA, PSP and CBD) separately with > 72% sensitivity, > 85% specificity and; AUC > 0.81.

The Early disease cohort

In the early disease cohort, blood NfL could distinguish patients with PD from patients with APD with 70% sensitivity and 80% specificity, AUC = 0.81, 95% CI

0.73-0.90. Similar results were seen when analyzing the different APD groups (MSA, PSP and CBD) separately with > 86% sensitivity, > 70% specificity and AUC > 0.80.

Correlations between blood NfL and clinical symptoms

The Lund cohort

In PD, increased levels of blood NfL correlated with longer disease duration ($\beta = 0.278$, $p < 0.001$) and more severe motor symptoms as measured by UPDRS III ($\beta = 0.227$, $p < 0.001$), Hoehn & Yahr ($\beta = 0.187$, $p = 0.004$), TUG ($\beta = 0.164$, $p = 0.036$), Tandem gait test ($\beta = 0.237$, $p = 0.045$) and LEDD ($\beta = 0.235$, $p = 0.003$). In APD blood NfL only correlated with UPDRS III ($\beta = 0.449$, $p = 0.001$) and Hoehn & Yahr ($\beta = 0.286$, $p = 0.040$).

The London cohort

Blood NfL did not correlate with disease duration or Hoehn & Yahr stage in neither PD nor APD in the London cohort.

The early cohort

Blood NfL did not correlate with disease duration or Hoehn & Yahr stage in neither PD nor APD in the Early cohort.

Correlations between blood NfL and white matter lesions

In the Lund cohort an increase in blood NfL correlated more white matter lesions as measured by Fazeka's score in PD ($R_s = 0.328$, $p = 0.002$) but not in controls or APD. The association in PD did not withstand correcting for age and gender.

Paper V

In this study we investigated levels of inflammatory biomarkers in CSF in PD, APD and healthy controls and if the inflammatory biomarkers correlated with disease severity in PD.

CSF inflammatory biomarker levels in the different diagnostic groups

There were significant correlations between age and inflammatory biomarkers in CSF (i.e. CRP, SAA, IL-8, YKL-40 and MCP-1) ($R_s \geq 0.186$, $p \leq 0.004$). Men had higher levels of MCP-1 compared with women ($p < 0.001$) but there were no other gender effects.

CSF levels of CRP and SAA were increased in PDD and MSA compared with controls and PD ($p \leq 0.018$). SAA was also higher in PSP compared with controls ($p = 0.014$). IL-6 was higher in PDD compared with controls ($p = 0.014$). CSF levels of IL-8 were higher in in all groups compared with controls ($p \leq 0.007$). PSP also had higher levels if IL8 compared with PD ($p = 0.021$) (Figure 15).

CSF levels of YKL-40 were lower in PD compared with controls ($p = 0.033$). Patients with MSA and PSP on the other hand had higher levels of YKL-40 compared with PD and PDD ($p \leq 0.026$) but not compared with controls. All corrected for age (Figure 15).

CSF levels of MCP-1 were higher in MSA compared with all other groups ($p \leq 0.048$), all corrected for age. Also correcting for gender did not change the results (Figure 15).

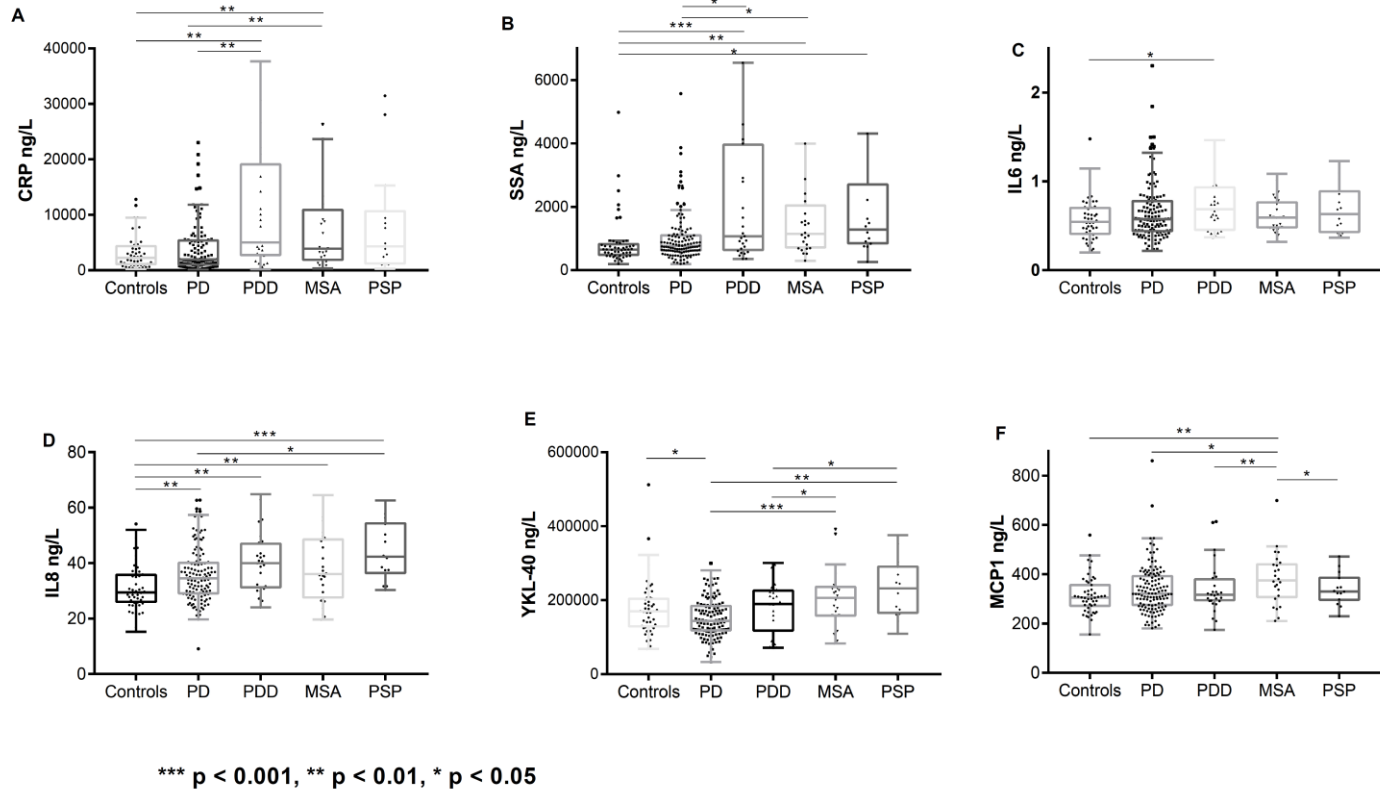


Figure 15. Box plots with scatter dots of inflammatory CSF biomarkers A) CRP, B) SAA, C) IL-6, D) IL-8, E) YKL-40 and F) MCP-1 in the different diagnostic groups presented as median and inter quartile range. Outer whiskers are 1.5 IQR. P values are from univariate general linear model adjusting for age. In the CRP figure, there were 4 outliers in PD and 2 in MSA outside the axis limit. In the SAA figure there were 5 outliers in PD, 3 in PDD, 2 in PSP and 2 in MSA outside the axis limit. In IL-6 there was 1 outlier in PDD outside the axis limit. Outliers outside the graph limits are included in all statistical analyses.

There were extensive correlations between the different inflammatory biomarkers in CSF in the cohort as a whole (Table 5). CRP and SAA correlated strongly with each other in the cohort as a whole but also in each individual diagnostic group ($\beta \geq 0.629$, $p \leq 0.001$). Further, CRP and SAA levels correlated with IL-6, IL-8 and YKL-40 in the cohort as a whole. The CSF levels of IL-6 correlated with IL-8 in the cohort as a whole, a correlation that remained in each patient diagnostic group but not controls ($\beta \geq 0.238$, $p \leq 0.002$). IL-8 correlated with YKL-40 and MCP-1. All corrected for age.

Table 5.

Correlations between different CSF biomarkers in the cohort as a whole, corrected for age, given as β values.

	YKL-40	MCP-1	CRP	SAA	IL-6	IL-8	α -syn	Tau	P-tau	A β ₄₂
MCP-1	NS									
CRP	0.131 *	NS								
SAA	0.137 *	NS	0.667 ***							
IL-6	NS	NS	0.138 *	0.178 **						
IL-8	0.242 ***	0.135 *	0.232 ***	0.220 ***	0.285 ***					
α-syn	0.393 ***	NS	NS	0.152 *	NS	0.203 **				
Tau	0.419 ***	NS	NS	0.147 *	- 0.175 **	NS	0.785 ***			
P-tau	0.348 ***	NS	NS	NS	NS	0.146 *	0.834 ***	0.746 ***		
Aβ₄₂	NS	NS	NS	NS	NS	NS	0.329 ***	NS	0.213 ***	
NfL	0.367 ***	0.207 **	0.177 *	0.250 ***	NS	0.292 ***	0.157 *	0.349 ***	NS	-0.126 *

* significant at <0.05. ** significant at <0.01. *** significant at < 0.001.

NS = not significant.

Correlations between inflammatory markers and clinical test scores in PD

Cognition

Inflammatory biomarkers, foremost CRP and SAA correlated cognitive impairment in PD with significant correlations between worse results on MMSE and CRP ($\beta = -0.164$, $p = 0.047$) and SAA ($\beta = -0.229$, $p = 0.005$). Similarly, AQT and Letter S fluency correlated with inflammatory markers (for AQT: CRP, $\beta = 0.214$, $p = 0.010$; SAA, $\beta = 0.208$, $p = 0.012$; and for Letter S fluency: SAA $\beta = -0.212$, $p = 0.010$; IL-6 $\beta = -0.190$, $p = 0.022$), all corrected for age.

Motor impairment

Inflammatory markers also correlated with motor impairment. A higher UPDRS III score correlated with higher levels of CRP ($\beta = 0.248$, $p = 0.003$) and SAA ($\beta = 0.261$, $p = 0.001$); and increased Hoehn & Yahr score correlated with increased levels of CRP ($\beta = 0.289$, $p < 0.001$), SAA ($\beta = 0.257$, $p = 0.002$) and IL-8 ($\beta = 0.168$, $p = 0.037$), all corrected for age.

Mood and fatigue

Depressive symptoms, measured by the depression items on HADS, as well as fatigue as measured by FACIT-f, correlated with CRP ($\beta = 0.263$, $p = 0.002$ and $\beta = 0.282$, $p < 0.001$, respectively) and SAA ($\beta = 0.333$ $p < 0.001$ and $\beta = 0.250$, $p = 0.003$, respectively). Furthermore, we also noted that IL-6 but not SAA or CRP correlated with lower score on HADS depression items in the control group ($\beta = -0.488$, $p = 0.018$). All corrected for age.

Discussion

Parkinson's disease is a quite common disease in the elderly population with great impact on the patient and their family but also to society. Still, the differential diagnosis can be difficult to make, especially early on in the disease course, with atypical parkinsonian disorders (i.e. MSA, PSP and CBD) being important differential diagnoses. In clinical trials and future treatments with disease modifying therapies directed against specific pathological mechanisms, an accurate and early diagnosis will be paramount since treatment most likely will be most effective if initiated before widespread neurodegeneration has occurred¹⁶⁶. Furthermore, to select the most appropriate treatment option, methods to assess the underlying neuropathology (or pathologies) causing the symptoms will most likely be highly important. Also, with an early and correct diagnosis symptomatic treatment can be initiated early and unnecessary investigations and medication errors can be avoided.

In the papers included in this thesis we have sought to find diagnostic as well as prognostic biomarkers to improve the differential diagnosis of PD and atypical parkinsonian disorders (APD) and to investigate the temporal changes in these biomarkers. Our aim has also been to further our understating of these disorders.

The discussion will include the more salient discussion points regarding the papers but also some general aspects of the cohort as a whole.

Improvement of the differential diagnosis

In paper I, where we investigate a panel of five CSF biomarkers in common dementias and parkinsonian disorders, we show that CSF NfL, a marker of neuroaxonal damage, is increased in APD compared with PD and controls, corroborating previous studies^{113, 145, 146, 148}. We also show that NfL in CSF can separate PD from APD with an accuracy (AUC = 0.93) high enough do give it clinical relevance. Even though NfL in CSF cannot help discriminate between the different atypical parkinsonian disorders, an increased NfL in CSF in a patient with parkinsonism indicates that further investigations are needed. Indeed, CSF NfL has become a tool in the clinical investigation of parkinsonian patients where

an atypical parkinsonism is suspected in is included in the Swedish national guidelines for PD.

The performance of lumbar puncture is however not always possible (for example in a primary care setting, or due to anticoagulant treatment or patient apprehension). The results from paper IV where we in independent cohorts show a good correlation between NfL in blood and NfL in CSF and that NfL in blood can distinguish between PD and APD with an accuracy comparable to NfL in CSF may thus prove highly useful in a clinical setting.

In paper I, confirming previous studies, we show that $A\beta_{42}$ is decreased and that tau and P-tau is increased in AD^{124, 133} and that $A\beta_{42}$ also is decreased in DLB¹⁹¹ reflecting the importance of amyloid pathology in DLB and PDD. However, we also show that $A\beta_{42}$ is even further decreased in AD compared with DLB. Also confirming previous studies^{102, 103, 105-109, 112, 113}, but contradicting others^{111, 112}, we show that α -syn is decreased in synucleinopathies (i.e. PD, PDD, DLB and MSA) compared with controls and patients with PSP and AD. Furthermore, we find that a combination of these biomarkers can separate PDD and DLB from AD with high accuracy (AUC = 0.90) with tau and α -syn contributing the most to the model. The improvement in the differential diagnosis between these dementias is highly important for the patients as treatment differs somewhat, foremost, patients with DLB are highly sensitive to neuroleptic medication. Even though α -syn is decreased in synucleinopathies including PD, the difference is subtle and overlap between the groups is still too large for α -syn to differentiate between PD and controls or other disorders on its own. There is thus still a need for further efforts in identifying new biomarkers for PD.

CSF biomarkers and neurodegeneration and disease progression

NfL is a biomarker of neuroaxonal damage. As shown in paper I and IV, NfL is increased in APD, diseases that usually are associated with a more aggressive disease course and more intense neurodegeneration. In paper I and IV we also show that NfL in both blood and CSF is associated with more severe parkinsonism as measured by Hoehn & Yahr stage in patients with PD. Furthermore we show that CSF NfL is associated with worse results on MMSE in AD. One can thus infer that NfL indeed is a marker of more aggressive disease with a higher rate of neurodegeneration. It is therefore somewhat surprising that we do not see a correlation between higher baseline levels of NfL and clinical progression in PD, neither for cognition nor motor progression, in paper II. This could possibly be due to the rather short follow-up. Furthermore, one can speculate that the non-

demented PD cohort investigated had not come to a stage where a more aggressive neurodegeneration becomes a major factor or that the patients with longer disease duration who have remained non-demented may have a more benign phenotype. On the other hand, in paper III we show that NfL increases in both early and later stages of the disease but that the rate of change of NfL levels did not correlate with disease progression.

In paper I we confirm that α -syn is decreased in synucleinopathies but increased in AD. Increased levels of α -syn have previously been linked to both AD and Creutzfeldt-Jakob, diseases with intense neurodegeneration^{103, 107}. Furthermore, in paper II we show that increased levels at baseline of α -syn within the PD group is associated with both cognitive decline and motor progression over 2 years confirming the result from one previous study¹¹⁴ but contradicting the results from another group¹⁰⁴. Moreover, in paper III we show that levels of α -syn increase during the later stages of the disease but not in the early stages. We thus hypothesize that α -syn is decreased early in the disease course, and probably also in the prodromal phase, as a reflection of an intracellular accumulation. However we also hypothesize that α -syn is bimodal and increases over time as a reflection of neuronal damage where α -syn is released to the extracellular space through neuronal cell death as its correlation with NfL and tau would suggest; or as a reflection of more complex mechanisms with an active secretion as a response to a physical stress²⁸ or defective clearance of the protein. Due to differences in study design, this bimodal temporal profile of α -syn may explain the divergent results from different studies. Indeed we find that all CSF biomarkers measured in paper II but A β ₄₂ (i.e. α -syn, tau, P-tau, NfL and YKL-40) increase over time but that α -syn, tau remain stable over the first 5 years of disease. In light of this it is interesting that α -syn does not seem to correlate with disease duration in cross sectional studies (including paper I and II in this thesis)^{102, 112}. This might be a reflection of the difficulty in estimating the disease duration retrospectively and may thus be imprecise when used on its own.

The association between tau and α -syn

Supporting results from another group, we show in paper II that baseline levels of α -syn strongly correlate with tau¹⁰⁶. In paper III, we also find that change in α -syn correlates with change in tau over 2 years. Parallel to α -syn, higher baseline levels of P-tau were associated with faster motor progression in paper II. Another group on the other hand has shown that the ratio P-tau/tau negatively correlates with the rate of change in UPDRS score over time¹²⁸. In paper III we find that a larger increase in P-tau over 2 years was associated with faster motor progression and

increased executive dysfunction as measured by Letter S fluency, but not any other cognitive test. Interestingly, one other group has also linked P-tau cognitive decline showing that higher baseline levels of P-tau were associated with the progression of both memory impairment and executive dysfunction¹²⁹. Tau thus seems to play a role not only in tauopathies but also in PD and interact with α -syn

Not only the α -syn-encoding gene (*SNCA*) but also the tau-encoding gene (*MAPT*) have been linked to PD through GWAS (genome-wide association studies)¹⁹². Tau has been found in the brains of sporadic PD patients^{193, 194}. Furthermore, P-tau has been found in the striatum of patients with PD and PDD^{194, 195}. Moreover, α -syn fibrils may induce aggregation of tau by cross-seeding¹⁹⁶. α -Syn may also disrupt the binding of tau to tubulin leading to cytoskeleton disorganization and tau aggregation, and has been shown to promote the phosphorylation of tau^{194, 197}. We hypothesize that a more aggressive disease with a greater synaptic degeneration may lead to higher levels of α -syn in the intracellular space which in turn may lead to a higher rate of phosphorylation of tau which may aggravate the disease further.

Cognitive decline

Cognitive impairment or dementia are common features in PD with a life-time incidence as high as 75-80%^{56, 57}. $A\beta_{42}$ is a biomarker associated with amyloid pathology. Neuropathological studies have shown that amyloid pathology is frequent in brains from patients with PDD^{198, 199}. Reduced CSF concentrations of $A\beta_{42}$ most likely reflect cortical amyloid accumulation^{200, 201}. Cross sectional studies have shown normal^{104, 113, 130} or decreased levels of $A\beta_{42}$ in PD and PDD compared with controls^{105, 106, 108, 126, 127, 131, 132}. Furthermore, low levels of $A\beta_{42}$ in non-demented PD patients have been shown to be associated with future cognitive decline and progression to PDD^{126, 134, 135}.

In paper I, we show decreased levels of $A\beta_{42}$ in in DLB but fail to find significantly lower levels in PD or PDD. In paper II we confirm that decreased levels of $A\beta_{42}$ in PD is associated with faster cognitive decline but only on episodic memory delayed recall, a cognitive domain more associated with AD than PD. Instead, increased levels of α -syn within the PD group were associated with worsening cognitive speed, a cognitive deficit more commonly associated with the cognitive profile in PDD. This confirms the results from another group who found that higher α -syn was associated with a higher rate of cognitive decline as measured by the Selective Reminding Test (SRT)-Total and SRT-Delayed (measures of verbal learning and memory), Symbol Digit Modalities Test (a test of visuospatial working memory/processing speed), and New Dot Test (visuospatial working memory)¹¹⁴. However, another study on biomarkers and cognitive decline

did not find an association between α -syn levels at baseline and cognitive decline as measured by MMSE and the Montreal Cognitive Assessment (MoCA)¹⁰⁴. However, MMSE and MoCA do not measure cognitive speed which might explain the differing results.

In paper III, we find that $A\beta_{42}$ remains stable over 2 years. This support results from studies on AD, showing that cortical amyloid accumulation and decrease in $A\beta_{42}$ occurs early in the disease process, developing years before symptoms occur^{124, 202, 203}. We also show that an increase in P-tau is associated with executive dysfunction as measured by Letter S fluency, confirming the results from a previous study¹²⁹. The pathophysiology of cognitive impairment in PD is thus likely different from the pathophysiology in AD. Furthermore, in paper III, we find that patients with long disease duration perform better on Letter S fluency compared with patients with short disease duration which might be an indication that patients who, in spite of a long disease duration, still have not developed cognitive dysfunction, are a separate clinical phenotype with better prognosis regarding cognition. This however needs to be investigated further but might be important to take into account when stratifying patient groups in future clinical trials.

Neuroinflammation in PD and APD

Inflammation, particularly microglia activation and astrocyte dysfunction have been heavily implicated in PD. However, evidence of microglia activation has also been seen in APD³¹⁻³⁴. In paper V, we find that inflammatory markers in CSF, foremost CRP and SAA and IL-8 are increased in PDD and MSA compared with PD and controls. This confirms and adds to the previously scarce studies on inflammatory biomarkers in CSF in parkinsonian disorders. In a study from our group, CRP in CSF has previously been shown to be increased in PDD compared with PD and controls¹⁶⁰. Another group has previously shown increased IL-6 in CSF in PD patients with cognitive impairment compared with PD without cognitive impairment and another study from our group found increased CSF IL-8 in PPD compared with controls^{159, 204}.

In paper V, we find correlations between YKL-40, SSA and IL-8 with α -syn as well tau and NfL which are markers of neuroaxonal injury. Furthermore, we find correlations between inflammation and more severe clinical symptoms both regarding cognition and motor symptoms in PD. This is in line with studies finding associations between microglia activation and worse results on MMSE in PDD^{205, 206}. In paper III we show that levels of YKL-40 increase over the 2 year follow up in PD and that the increase of YKL-40 is associated with both motor

progression and cognitive decline. Inflammation thus seems to be associated with a more aggressive disease in PD. One can speculate that α -syn pathology triggers an inflammatory response that may aggravate the disease and neurodegenerative process even further.

In paper V, we find that YKL-40, a biomarker for mainly astrocytes and microglia, is decreased in PD compared with controls and APD. This is in line with one previous study¹⁵⁶ but contrasts others^{113, 155}. Furthermore, we see strong correlations between α -syn and YKL-40 in paper V and between the increases in α -syn levels with the increase in YKL-40 in paper III. In PD, astrocytes have been suggested to have a protective role^{37, 38}. On the other hand, α -syn positive astrocytes have been seen to correlate with cell death in PD³⁹ indicating that α -syn pathology might lead to impaired astrocyte function. Astrocyte dysfunction in PD might thus be reflected by a down regulation of YKL-40. In APD we find increased levels of YKL-40, which corroborates previous results^{113, 156}. The increase of YKL-40 in APD may be a reflection of astrocyte activation as a response to the injury the neurodegeneration entails. Indeed, increase in YKL-40 is associated with an increase in α -syn (due to an increased release or defective clearance) and to neuroaxonal injury as reflected by the association with tau and NfL.

Neuroinflammation and mood in PD

In paper V we also find that CSP and SAA are associated with depression and fatigue in PD but not in controls, corroborating and adding to previous results¹⁶⁰. Moreover, in line with some previous investigations but contradicting others, we find that lower IL-6 correlates with depressive mood in controls indicating different mechanisms in the pathophysiology of the non-motor symptom depression in PD^{160, 207, 208}.

Pre-analytic handling of CSF samples

Can pre-analytic handling of CSF-samples affect the results in biomarker studies?

Diurnal variations of biomarker levels have been discussed in several studies. Although one study has shown a large diurnal variation of $A\beta_{42}$ in healthy subjects²⁰⁹, most studies have shown no significant fluctuations in tau, P-tau or $A\beta_{42}$ ^{210, 211}. A few studies on α -syn have shown slight or no fluctuations²¹². Thus, as CSF samples for non-emergency conditions generally are taken during the day,

standardization is not needed. It is however recommended to record sampling time and to take blood and CSF samples at the same time²¹³.

Studies on traditional AD biomarkers have shown no gradient effect. However, CSF α -syn has been found to show slightly decreased levels in a rostro-caudal gradient. A standardized volume is therefore recommended, generally 12 ml²¹³. As a rule, in our cohort we tap 20 ml.

More importantly, α -syn and A β peptides bind to the walls of non-polypropylene tubes²¹⁰. Therefore polypropylene tubes should be used²¹³.

Although several studies have shown stable levels of A β ₄₂, tau and P-tau at room temperature, in one case up to 5 days, there are conflicting results^{213, 214}. Furthermore, α -syn has been shown to decrease by 40% when stored at 4 °C for 4 days²¹³. The number of freeze/thaw cycles should be limited as this can affect concentrations α -syn and A β ₄₂ and possibly also tau^{213, 214}. Studies have indicated that freezing temperature can affect the levels tau and P-tau with decreased levels when stored at -20° compared with -80°. Furthermore, A β ₄₂ has been shown to remain stable over 2 years when stored at -80°²¹⁰. We therefore store CSF and blood samples at -80° pending analysis.

In this study, we have taken great care to use a standardized handling of or samples according to the Alzheimer's association flow chart¹³³. However, small differences in pre-analytic handling may account for differences in CSF levels between sites in paper I and IV.

Confounders

As evident in this thesis, choices need to be made regarding what confounding factors to correct for in biomarker studies. In the different papers for this thesis, different choices have been made based upon the effects on confounding factors found in the individual study as well as the current knowledge at the time.

LEDD: Even though much discussed as a potential confounder, α -syn has over the last few years been shown to be consistently decreased in De Novo patients^{102, 106, 110}, similar to studies on PD patients currently on medication^{105, 107}. Dopaminergic medication thus does not seem to affect levels of CSF α -syn. Furthermore, dopaminergic treatment also does not seem to affect the levels of tau, P-tau or A β ₄₂²¹⁵. A correlation between LEDD and CSF biomarker levels may thus be a reflection of increased disease severity rather than an effect of levodopa treatment.

Gender: Most studies have found no gender effects of α -syn in PD although there are some conflicting results. In the papers for this thesis we have not found any

gender-effects of α -syn. There is thus not enough evidence to consider gender a confounder in CSF studies on α -syn²¹⁵. On the other hand, we saw an increase of blood NfL in women compared with men in paper IV and subsequently corrected for gender in the analyses in that study. In CSF however, gender has not been shown to affect NfL concentrations, when commented at all in publications¹⁴⁶.

Age: Results have been conflicting regarding α -syn and age²¹⁵. However, we have seen correlations between α -syn and age in the papers of this thesis^{216, 217}. Even though results are also conflicting regarding tau, P-tau and A β ₄₂ some studies show an effect of age²¹⁵. Furthermore, NfL has been shown to increase with age¹⁴⁶ and this is also something we find in the papers for this thesis²¹⁶⁻²¹⁸. Indeed, clinical cut-off levels for CSF NfL are increased with an increased age. We have subsequently chosen to adjust for age in our studies.

Limitations

In our cohort, we follow our patients over time and use diagnostic criteria to ensure as correct a diagnosis as possible. However, for a definite diagnosis neuropathology is required. The lack of neuropathology, but for a few cases in our cohort, is therefore a major limitation.

The strict use of criteria may ensure a higher specificity but may also lead to a loss in sensitivity particularly in identifying patients with PSP. The criteria we use mainly identify PSP-RS and we may therefore miss other subgroups of PSP.

Due to the heterogeneous nature of these diseases and the slow progression rate in PD, a large cohort with long follow-up would improve the study. In the longitudinal studies of this thesis we have only 2 years follow-up data. This is too short to draw any definite conclusions about clinical progression and temporal changes in CSF biomarkers over the long term. We are therefore extending our follow-up to 10 years.

In the papers included in this thesis we have only studied total α -syn. To further improve our understanding of the role of α -syn, future longitudinal studies should also include phosphorylated, truncated and oligomeric forms of α -syn.

In our cohort, the patients are examined in the ON state. We believe that this improves our possibility to continue to follow the patients over time as abstaining from medication may be increasingly inconvenient for the PD patient as the disease progresses. However, this might reduce the reliability of the different motor tests (possibly except Hoehn & Yahr stage) used in the study. Also, any clinical test is a snapshot in time and might be influenced by a multitude of

different individual factors. It is therefore important to include large numbers of patients to increase the reliability of the results. Also, results need to be replicated.

Still, we lose some, mainly late stage, patients to follow-up. It may be too much of a strain to come to the clinic or they don't have relatives who can help them. Patients in our cohort, foremost those with long disease duration, may therefore not be fully representative of the disease population.

Even though patients in our cohort are examined by only a few medical doctors, inter rater variability may affect result on clinical test scores. This may be particularly problematic when collaborating between different sites.

The cross sectional design of paper V makes it impossible to address if inflammation, microglia activation and astrocyte dysfunction is detrimental or beneficial. Future longitudinal studies are therefore needed.

Future directions

Further longitudinal studies are needed. To address whether inflammation with microglia activation and astrocyte dysfunction truly is detrimental as hypothesized, longitudinal studies need to be performed. Also, to further investigate the temporal changes in CSF biomarkers, further longitudinal studies over a longer period of time are needed. With a follow-up up to ten years we will be able to address these issues to some extent.

There is also a need of better biomarkers for PD and related pathologies. For example, α -syn measured using existing assays is not Lewy body-specific; α -syn is a protein released by normal synapses and also abundantly expressed in non-neuronal cell types, e.g. erythrocytes. A Lewy body-specific or -enriched α -syn form would be a promising biomarker candidate to explore further. Such forms would most likely be present at very low concentrations in biofluids but could potentially be quantified using novel ultrasensitive technology such as Simoa.

With new imaging possibilities we are now also investigating patients with Tau-PET, hoping this will further contribute to our understanding of the role of tau in the tauopathies as well as in Parkinson's disease with dementia.

In spite of the effort by our group and many others, there is still not one biomarker that can diagnose Parkinson's disease with a high enough degree of diagnostic accuracy. The use of diagnostic criteria requiring motor symptoms is still the golden standard. For clinical trials this is of course unsatisfactory as disease-modifying therapies are likely most effective were they to be initiated early, before major neuronal loss has already occurred. Further efforts to identify new

biomarkers are therefore needed. Ideally this biomarker should be easy to use in a primary care setting, helping the non-expert make an early and accurate diagnosis as well as making screenings of patients with prodromal symptoms possible for early intervention clinical trials. However, most likely the key to an early diagnosis will be a combination of biomarkers, probably multimodal, making use of a combination of different techniques in patients with an increased risk such as patients with hyposmia and REM sleep behavior disorder.

Conclusions

The levels of NfL are increased in both blood and CSF in patients with Atypical parkinsonian disorders (APD) compared with Parkinson's disease (PD) and controls. Furthermore NfL in both blood and CSF can differentiate between PD and APD with a relatively high diagnostic accuracy. This may prove highly useful in the preliminary investigation of patients with parkinsonism, even outside highly specialist settings.

We confirm that α -syn is decreased in PD, possibly because of intraneuronal accumulation of the protein (the latter remains an unproven hypothesis). However we show that increased levels of α -syn at baseline within the PD group are associated with increased motor progression as well as increased cognitive decline. Furthermore, α -syn correlates with markers of neurodegeneration (tau and NfL) suggesting an association between α -syn and neurodegeneration. Also, low levels of $A\beta_{42}$ are associated with subsequent memory decline in PD.

Over a follow-up of two years, CSF levels of α -syn, tau, P-tau, NfL and YKL-40 but not $A\beta_{42}$ increase in patients with PD but remain stable in controls. However α -syn and tau remained stable in patients early in the disease course and increased only in the group with > 5 years disease duration, suggesting that the increase in α -syn is a reflection of a more intense neurodegeneration in patients with longer disease duration.

We also confirm the role of neuroinflammation in PD. We find increased levels of inflammatory biomarkers in PDD and MSA compared with PD and controls. YKL-40 was however decreased in PD suggesting astrocyte dysfunction. Furthermore, inflammatory biomarkers correlate with more motor symptoms as well as cognitive impairment in PD suggesting an association between inflammation and a more aggressive disease.

Acknowledgement

I want to express my deepest gratitude to all of you who have offered help and support during my research studies. Without you, completing this thesis would not have been possible.

First, I would like to thank my supervisor Oskar Hansson, for presenting this exciting, clinical research project. Thank you for sharing your scientific knowledge. Thank you also for your patience and for being easy to reach for numerous questions. Foremost, I want to thank you for your enthusiasm and endless amount of ideas.

To my co-supervisors: Henrik Zetterberg, for your endless enthusiasm and knowledge on biomarkers and neurodegeneration. Håkan Widner, for your warm support and clinical expertise.

To my former co-supervisors: Christer Nilsson, for your help and knowledge in atypical parkinsonism. Peter Hagell, for your expertise in clinical rating scales.

To Yulia Surova, my colleague, roommate and friend, for sharing this journey. I wish you all the best during these last months leading up to your dissertation!

To Daniel Lindqvist, for contributing a psychiatric perspective and for your help and support in statistics.

To Shorena Janelidze, for your support and patience. Thank you also for helping me make better figures.

To my other research friends and colleagues, Ruben Smith, Sebastian Palmqvist, Niklas Mattsson and Mattis Jalakas for discussions and friendship.

To the Department of Neurology, for giving me the opportunity to share my time between clinical work and research. And to my colleagues at the department of neurology, for your support and friendship.

To the research nurses, Jan Reimer, Ann Johansson and Katarina Johansson: for your warm support. Thank you for organizing the administration in this study with a growing amount of data! Thank you for your invaluable work in the clinical investigations of the patients in this study.

To all the patients and their families for their participation in the study. Thank you for your great contribution.

To Andrea Nord, for friendship and music. Thank you for your help with proofreading.

To my parents Elisabet and Håkan Hall for your endless love and support. Thank you for discussions on life as well scientific research. To my brother Ola and to Karin for your love and for always being there.

To my extended family, Olof; Lisa and Peter; Mia and Mattias, for your friendship and love.

To Henrik, my husband and best friend. For your love, support and understanding. For always being there no matter what.

And finally, to my beloved children Linn and Joel. You are my miracles and a great source of joy in my life.

References

1. Parkinson J. An essay on the shaking palsy. 1817. *The Journal of neuropsychiatry and clinical neurosciences*. 2002;14:223-236; discussion 222
2. Parkinson J. An essay on the shaking palsy. *Whittingham and Rowland for Sherwood, Needly and Jones, London*. 1817
3. Charcot J-M. De la paralysie agitante. In *Oeuvres Complètes (t 1) Leçons sur les maladies du système nerveux*, pp. 155–188 A Delahaye, Paris. 1872
4. de Lau LM, Breteler MM. Epidemiology of parkinson's disease. *The Lancet Neurology*. 2006;5:525-535
5. Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R. How common are the "common" neurologic disorders? *Neurology*. 2007;68:326-337
6. Wirdefeldt K, Adami HO, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of parkinson's disease: A review of the evidence. *Eur J Epidemiol*. 2011;26 Suppl 1:S1-58
7. Linder J, Stenlund H, Forsgren L. Incidence of parkinson's disease and parkinsonism in northern sweden: A population-based study. *Movement disorders : official journal of the Movement Disorder Society*. 2010;25:341-348
8. Lökk J, Borg S, Svensson J, Persson U, Ljunggren G. Drug and treatment costs in parkinson's disease patients in sweden. *Acta Neurol Scand*. 2012;125:142-147
9. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic parkinson's disease. *Neurobiology of aging*. 2003;24:197-211
10. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in lewy bodies. *Nature*. 1997;388:839-840
11. Jellinger KA. Neuropathology of sporadic parkinson's disease: Evaluation and changes of concepts. *Movement disorders : official journal of the Movement Disorder Society*. 2012;27:8-30
12. Schulz-Schaeffer WJ. The synaptic pathology of alpha-synuclein aggregation in dementia with lewy bodies, parkinson's disease and parkinson's disease dementia. *Acta neuropathologica*. 2010;120:131-143
13. Braak H, Muller CM, Rub U, Ackermann H, Bratzke H, de Vos RA, et al. Pathology associated with sporadic parkinson's disease--where does it end? *Journal of neural transmission. Supplementum*. 2006:89-97
14. Jellinger KA. A critical evaluation of current staging of alpha-synuclein pathology in lewy body disorders. *Biochimica et biophysica acta*. 2009;1792:730-740

15. Beach TG, Adler CH, Lue L, Sue LI, Bachalakuri J, Henry-Watson J, et al. Unified staging system for lewy body disorders: Correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. *Acta neuropathologica*. 2009;117:613-634
16. Brundin P, Li JY, Holton JL, Lindvall O, Revesz T. Research in motion: The enigma of parkinson's disease pathology spread. *Nat Rev Neurosci*. 2008;9:741-745
17. Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, et al. Lewy bodies in grafted neurons in subjects with parkinson's disease suggest host-to-graft disease propagation. *Nat Med*. 2008;14:501-503
18. Dickson DW, Fujishiro H, DelleDonne A, Menke J, Ahmed Z, Klos KJ, et al. Evidence that incidental lewy body disease is pre-symptomatic parkinson's disease. *Acta neuropathologica*. 2008;115:437-444
19. Schrag A, Horsfall L, Walters K, Noyce A, Petersen I. Prediagnostic presentations of parkinson's disease in primary care: A case-control study. *The Lancet. Neurology*. 2015;14:57-64
20. Del Tredici K, Braak H. Review: Sporadic parkinson's disease: Development and distribution of alpha-synuclein pathology. *Neuropathology and applied neurobiology*. 2016;42:33-50
21. Adler CH, Beach TG. Neuropathological basis of nonmotor manifestations of parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 2016
22. DeLong MR, Wichmann T. Basal ganglia circuits as targets for neuromodulation in parkinson disease. *JAMA Neurol*. 2015;72:1354-1360
23. Halliday GM, Leverenz JB, Schneider JS, Adler CH. The neurobiological basis of cognitive impairment in parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 2014;29:634-650
24. Barnum CJ, Tansey MG. Modeling neuroinflammatory pathogenesis of parkinson's disease. *Progress in brain research*. 2010;184:113-132
25. Yan J, Fu Q, Cheng L, Zhai M, Wu W, Huang L, et al. Inflammatory response in parkinson's disease (review). *Molecular medicine reports*. 2014;10:2223-2233
26. Wojtera M, Sikorska B, Sobow T, Liberski PP. Microglial cells in neurodegenerative disorders. *Folia neuropathologica / Association of Polish Neuropathologists and Medical Research Centre, Polish Academy of Sciences*. 2005;43:311-321
27. Ouchi Y, Yagi S, Yokokura M, Sakamoto M. Neuroinflammation in the living brain of parkinson's disease. *Parkinsonism & related disorders*. 2009;15 Suppl 3:S200-204
28. Lim S, Chun Y, Lee JS, Lee SJ. Neuroinflammation in synucleinopathies. *Brain pathology*. 2016;26:404-409
29. Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience*. 1990;39:151-170

30. Gerhard A, Pavese N, Hotton G, Turkheimer F, Es M, Hammers A, et al. In vivo imaging of microglial activation with [11c](r)-pk11195 pet in idiopathic parkinson's disease. *Neurobiology of disease*. 2006;21:404-412
31. Gerhard A, Trender-Gerhard I, Turkheimer F, Quinn NP, Bhatia KP, Brooks DJ. In vivo imaging of microglial activation with [11c](r)-pk11195 pet in progressive supranuclear palsy. *Movement disorders : official journal of the Movement Disorder Society*. 2006;21:89-93
32. Gerhard A, Watts J, Trender-Gerhard I, Turkheimer F, Banati RB, Bhatia K, et al. In vivo imaging of microglial activation with [11c](r)-pk11195 pet in corticobasal degeneration. *Movement disorders : official journal of the Movement Disorder Society*. 2004;19:1221-1226
33. Ishizawa K, Komori T, Sasaki S, Arai N, Mizutani T, Hirose T. Microglial activation parallels system degeneration in multiple system atrophy. *Journal of neuropathology and experimental neurology*. 2004;63:43-52
34. Ishizawa K, Dickson DW. Microglial activation parallels system degeneration in progressive supranuclear palsy and corticobasal degeneration. *Journal of neuropathology and experimental neurology*. 2001;60:647-657
35. Vieira BD, Radford RA, Chung RS, Guillemin GJ, Pountney DL. Neuroinflammation in multiple system atrophy: Response to and cause of alpha-synuclein aggregation. *Frontiers in cellular neuroscience*. 2015;9:437
36. Teismann P, Schulz JB. Cellular pathology of parkinson's disease: Astrocytes, microglia and inflammation. *Cell Tissue Res*. 2004;318:149-161
37. Damier P, Hirsch EC, Zhang P, Agid Y, Javoy-Agid F. Glutathione peroxidase, glial cells and parkinson's disease. *Neuroscience*. 1993;52:1-6
38. Colombo E, Farina C. Astrocytes: Key regulators of neuroinflammation. *Trends Immunol*. 2016;37:608-620
39. Wakabayashi K, Hayashi S, Yoshimoto M, Kudo H, Takahashi H. Nacp/alpha-synuclein-positive filamentous inclusions in astrocytes and oligodendrocytes of parkinson's disease brains. *Acta neuropathologica*. 2000;99:14-20
40. Hernandez DG, Reed X, Singleton AB. Genetics in parkinson disease: Mendelian versus non-mendelian inheritance. *Journal of neurochemistry*. 2016
41. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with parkinson's disease. *Science*. 1997;276:2045-2047
42. Puschmann A. Monogenic parkinson's disease and parkinsonism: Clinical phenotypes and frequencies of known mutations. *Parkinsonism & related disorders*. 2013;19:407-415
43. Lill CM. Genetics of parkinson's disease. *Mol Cell Probes*. 2016;30:386-396
44. Klein C, Westenberger A. Genetics of parkinson's disease. *Cold Spring Harb Perspect Med*. 2012;2:a008888
45. Wirdefeldt K, Gatz M, Reynolds CA, Prescott CA, Pedersen NL. Heritability of parkinson disease in swedish twins: A longitudinal study. *Neurobiology of aging*. 2011;32:1923 e1921-1928
46. Jankovic J. Parkinson's disease: Clinical features and diagnosis. *Journal of neurology, neurosurgery, and psychiatry*. 2008;79:368-376

47. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic parkinson's disease: A clinico-pathological study of 100 cases. *Journal of neurology, neurosurgery, and psychiatry*. 1992;55:181-184
48. Berg D, Postuma RB, Adler CH, Bloem BR, Chan P, Dubois B, et al. Mds research criteria for prodromal parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 2015;30:1600-1611
49. Chaudhuri KR, Healy DG, Schapira AH, National Institute for Clinical E. Non-motor symptoms of parkinson's disease: Diagnosis and management. *The Lancet. Neurology*. 2006;5:235-245
50. Santos-Garcia D, de la Fuente-Fernandez R. Impact of non-motor symptoms on the quality of life of patients with parkinson's disease: Some questions beyond research findings. *Journal of the neurological sciences*. 2013;335:239
51. He L, Lee EY, Sterling NW, Kong L, Lewis MM, Du G, et al. The key determinants to quality of life in parkinson's disease patients: Results from the parkinson's disease biomarker program (pdbp). *Journal of Parkinson's disease*. 2016
52. Lang AE. A critical appraisal of the premotor symptoms of parkinson's disease: Potential usefulness in early diagnosis and design of neuroprotective trials. *Movement disorders : official journal of the Movement Disorder Society*. 2011;26:775-783
53. Noyce AJ, Lees AJ, Schrag AE. The prediagnostic phase of parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry*. 2016
54. Boeve BF. Mild cognitive impairment associated with underlying alzheimer's disease versus lewy body disease. *Parkinsonism & related disorders*. 2012;18 Suppl 1:S41-44
55. Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, et al. Clinical diagnostic criteria for dementia associated with parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 2007;22:1689-1707; quiz 1837
56. Svenningsson P, Westman E, Ballard C, Aarsland D. Cognitive impairment in patients with parkinson's disease: Diagnosis, biomarkers, and treatment. *The Lancet. Neurology*. 2012;11:697-707
57. Aarsland D, Kurz MW. The epidemiology of dementia associated with parkinson's disease. *Brain pathology*. 2010;20:633-639
58. Petrova M, Mehrabian-Spasova S, Aarsland D, Raycheva M, Traykov L. Clinical and neuropsychological differences between mild parkinson's disease dementia and dementia with lewy bodies. *Dementia and geriatric cognitive disorders extra*. 2015;5:212-220
59. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for parkinson disease. *Archives of neurology*. 1999;56:33-39
60. Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, et al. Mds clinical diagnostic criteria for parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 2015;30:1591-1601
61. Fanciulli A, Wenning GK. Multiple-system atrophy. *The New England journal of medicine*. 2015;372:1375-1376

62. Stefanova N, Wenning GK. Review: Multiple system atrophy: Emerging targets for interventional therapies. *Neuropathology and applied neurobiology*. 2016;42:20-32
63. Jellinger KA, Wenning GK. Multiple system atrophy: Pathogenic mechanisms and biomarkers. *Journal of neural transmission*. 2016;123:555-572
64. Gilman S, Low PA, Quinn N, Albanese A, Ben-Shlomo Y, Fowler CJ, et al. Consensus statement on the diagnosis of multiple system atrophy. *Journal of the neurological sciences*. 1999;163:94-98
65. Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, et al. Second consensus statement on the diagnosis of multiple system atrophy. *Neurology*. 2008;71:670-676
66. Ling H. Clinical approach to progressive supranuclear palsy. *Journal of movement disorders*. 2016;9:3-13
67. Steele JC, Richardson JC, Olszewski J. Progressive supranuclear palsy. A heterogeneous degeneration involving the brain stem, basal ganglia and cerebellum with vertical gaze and pseudobulbar palsy, nuchal dystonia and dementia. *Archives of neurology*. 1964;10:333-359
68. Litvan I, Mangone CA, McKee A, Verny M, Parsa A, Jellinger K, et al. Natural history of progressive supranuclear palsy (steele-richardson-olszewski syndrome) and clinical predictors of survival: A clinicopathological study. *Journal of neurology, neurosurgery, and psychiatry*. 1996;60:615-620
69. Respondek G, Hoglinger GU. The phenotypic spectrum of progressive supranuclear palsy. *Parkinsonism & related disorders*. 2016;22 Suppl 1:S34-36
70. Williams DR, Holton JL, Strand C, Pittman A, de Silva R, Lees AJ, et al. Pathological tau burden and distribution distinguishes progressive supranuclear palsy-parkinsonism from richardson's syndrome. *Brain : a journal of neurology*. 2007;130:1566-1576
71. Williams DR, Lees AJ. Progressive supranuclear palsy: Clinicopathological concepts and diagnostic challenges. *The Lancet. Neurology*. 2009;8:270-279
72. Litvan I, Agid Y, Jankovic J, Goetz C, Brandel JP, Lai EC, et al. Accuracy of clinical criteria for the diagnosis of progressive supranuclear palsy (steele-richardson-olszewski syndrome). *Neurology*. 1996;46:922-930
73. Armstrong MJ, Litvan I, Lang AE, Bak TH, Bhatia KP, Borroni B, et al. Criteria for the diagnosis of corticobasal degeneration. *Neurology*. 2013;80:496-503
74. Kouri N, Murray ME, Hassan A, Rademakers R, Uitti RJ, Boeve BF, et al. Neuropathological features of corticobasal degeneration presenting as corticobasal syndrome or richardson syndrome. *Brain : a journal of neurology*. 2011;134:3264-3275
75. Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A, Logroscino G. Accuracy of clinical diagnosis of parkinson disease: A systematic review and meta-analysis. *Neurology*. 2016;86:566-576
76. Joutsa J, Gardberg M, Roytta M, Kaasinen V. Diagnostic accuracy of parkinsonism syndromes by general neurologists. *Parkinsonism & related disorders*. 2014;20:840-844

77. Hughes AJ, Daniel SE, Ben-Shlomo Y, Lees AJ. The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. *Brain : a journal of neurology*. 2002;125:861-870
78. Brooks DJ. Molecular imaging of dopamine transporters. *Ageing Res Rev*. 2016;30:114-121
79. Marek KL, Seibyl JP, Zoghbi SS, Zea-Ponce Y, Baldwin RM, Fussell B, et al. [123i] beta-cit/spect imaging demonstrates bilateral loss of dopamine transporters in hemi-parkinson's disease. *Neurology*. 1996;46:231-237
80. Marek K, Innis R, van Dyck C, Fussell B, Early M, Eberly S, et al. [123i]beta-cit spect imaging assessment of the rate of parkinson's disease progression. *Neurology*. 2001;57:2089-2094
81. Ba F, Martin WR. Dopamine transporter imaging as a diagnostic tool for parkinsonism and related disorders in clinical practice. *Parkinsonism & related disorders*. 2015;21:87-94
82. Politis M. Neuroimaging in parkinson disease: From research setting to clinical practice. *Nature reviews. Neurology*. 2014;10:708-722
83. Jakobson Mo S, Linder J, Forsgren L, Holmberg H, Larsson A, Riklund K. Pre- and postsynaptic dopamine spect in idiopathic parkinsonian diseases: A follow-up study. *Biomed Res Int*. 2013;2013:143532
84. Weingarten CP, Sundman MH, Hickey P, Chen NK. Neuroimaging of parkinson's disease: Expanding views. *Neurosci Biobehav Rev*. 2015;59:16-52
85. Arena JE, Stoessl AJ. Optimizing diagnosis in parkinson's disease: Radionuclide imaging. *Parkinsonism & related disorders*. 2016;22 Suppl 1:S47-51
86. Loane C, Politis M. Positron emission tomography neuroimaging in parkinson's disease. *Am J Transl Res*. 2011;3:323-341
87. Schwarz ST, Afzal M, Morgan PS, Bajaj N, Gowland PA, Auer DP. The 'swallow tail' appearance of the healthy nigrosome - a new accurate test of parkinson's disease: A case-control and retrospective cross-sectional mri study at 3t. *PLoS one*. 2014;9:e93814
88. Reiter E, Mueller C, Pinter B, Krismer F, Scherfler C, Esterhammer R, et al. Dorsolateral nigral hyperintensity on 3.0t susceptibility-weighted imaging in neurodegenerative parkinsonism. *Movement disorders : official journal of the Movement Disorder Society*. 2015;30:1068-1076
89. Bae YJ, Kim JM, Kim E, Lee KM, Kang SY, Park HS, et al. Loss of nigral hyperintensity on 3 tesla mri of parkinsonism: Comparison with (123) i-fp-cit spect. *Movement disorders : official journal of the Movement Disorder Society*. 2016;31:684-692
90. Kim JM, Jeong HJ, Bae YJ, Park SY, Kim E, Kang SY, et al. Loss of substantia nigra hyperintensity on 7 tesla mri of parkinson's disease, multiple system atrophy, and progressive supranuclear palsy. *Parkinsonism & related disorders*. 2016;26:47-54
91. Pilotto A, Yilmaz R, Berg D. Developments in the role of transcranial sonography for the differential diagnosis of parkinsonism. *Curr Neurol Neurosci Rep*. 2015;15:43

92. Dabrowska M, Schinwelski M, Sitek EJ, Muraszko-Klaudiel A, Brockhuis B, Jamrozik Z, et al. The role of neuroimaging in the diagnosis of the atypical parkinsonian syndromes in clinical practice. *Neurol Neurochir Pol.* 2015;49:421-431
93. Surova Y, Szczepankiewicz F, Latt J, Nilsson M, Eriksson B, Leemans A, et al. Assessment of global and regional diffusion changes along white matter tracts in parkinsonian disorders by mr tractography. *PLoS one.* 2013;8:e66022
94. Surova Y, Nilsson M, Latt J, Lampinen B, Lindberg O, Hall S, et al. Disease-specific structural changes in thalamus and dentatorubrothalamic tract in progressive supranuclear palsy. *Neuroradiology.* 2015;57:1079-1091
95. Teune LK, Renken RJ, Mudali D, De Jong BM, Dierckx RA, Roerdink JB, et al. Validation of parkinsonian disease-related metabolic brain patterns. *Movement disorders : official journal of the Movement Disorder Society.* 2013;28:547-551
96. Garraux G, Phillips C, Schrouff J, Kreisler A, Lemaire C, Degueldre C, et al. Multiclass classification of fdg pet scans for the distinction between parkinson's disease and atypical parkinsonian syndromes. *Neuroimage Clin.* 2013;2:883-893
97. Calo L, Wegrzynowicz M, Santivanez-Perez J, Grazia Spillantini M. Synaptic failure and alpha-synuclein. *Movement disorders : official journal of the Movement Disorder Society.* 2016;31:169-177
98. Fauvet B, Mbefo MK, Fares MB, Desobry C, Michael S, Ardah MT, et al. Alpha-synuclein in central nervous system and from erythrocytes, mammalian cells, and escherichia coli exists predominantly as disordered monomer. *J Biol Chem.* 2012;287:15345-15364
99. Bartels T, Choi JG, Selkoe DJ. Alpha-synuclein occurs physiologically as a helically folded tetramer that resists aggregation. *Nature.* 2011;477:107-110
100. Burre J, Sharma M, Sudhof TC. Alpha-synuclein assembles into higher-order multimers upon membrane binding to promote snare complex formation. *Proc Natl Acad Sci U S A.* 2014;111:E4274-4283
101. Ingelsson M. Alpha-synuclein oligomers-neurotoxic molecules in parkinson's disease and other lewy body disorders. *Front Neurosci.* 2016;10:408
102. Hong Z, Shi M, Chung KA, Quinn JF, Peskind ER, Galasko D, et al. Dj-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of parkinson's disease. *Brain : a journal of neurology.* 2010;133:713-726
103. Mollenhauer B, Locascio JJ, Schulz-Schaeffer W, Sixel-Doring F, Trenkwalder C, Schlossmacher MG. Alpha-synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: A cohort study. *The Lancet. Neurology.* 2011;10:230-240
104. Parnetti L, Farotti L, Eusebi P, Chiasserini D, De Carlo C, Giannandrea D, et al. Differential role of csf alpha-synuclein species, tau, and abeta42 in parkinson's disease. *Front Aging Neurosci.* 2014;6:53
105. Shi M, Bradner J, Hancock AM, Chung KA, Quinn JF, Peskind ER, et al. Cerebrospinal fluid biomarkers for parkinson disease diagnosis and progression. *Annals of neurology.* 2011;69:570-580
106. Kang JH, Irwin DJ, Chen-Plotkin AS, Siderowf A, Caspell C, Coffey CS, et al. Association of cerebrospinal fluid beta-amyloid 1-42, t-tau, p-tau181, and alpha-

- synuclein levels with clinical features of drug-naïve patients with early parkinson disease. *JAMA Neurol.* 2013;70:1277-1287
107. Mollenhauer B, Cullen V, Kahn I, Krastins B, Outeiro TF, Pepivani I, et al. Direct quantification of csf alpha-synuclein by elisa and first cross-sectional study in patients with neurodegeneration. *Exp Neurol.* 2008;213:315-325
 108. Skogseth RE, Bronnick K, Pereira JB, Mollenhauer B, Weintraub D, Fladby T, et al. Associations between cerebrospinal fluid biomarkers and cognition in early untreated parkinson's disease. *Journal of Parkinson's disease.* 2015;5:783-792
 109. Zetterberg H, Petzold M, Magdalinou N. Cerebrospinal fluid alpha-synuclein levels in parkinson's disease--changed or unchanged? *European journal of neurology.* 2014;21:365-367
 110. Mollenhauer B, Trautmann E, Taylor P, Manninger P, Sixel-Doring F, Ebentheuer J, et al. Total csf alpha-synuclein is lower in de novo parkinson patients than in healthy subjects. *Neurosci Lett.* 2013;532:44-48
 111. Ohrfelt A, Grognet P, Andreasen N, Wallin A, Vanmechelen E, Blennow K, et al. Cerebrospinal fluid alpha-synuclein in neurodegenerative disorders-a marker of synapse loss? *Neurosci Lett.* 2009;450:332-335
 112. Parnetti L, Chiasserini D, Bellomo G, Giannandrea D, De Carlo C, Qureshi MM, et al. Cerebrospinal fluid tau/alpha-synuclein ratio in parkinson's disease and degenerative dementias. *Movement disorders : official journal of the Movement Disorder Society.* 2011;26:1428-1435
 113. Magdalinou NK, Paterson RW, Schott JM, Fox NC, Mummery C, Blennow K, et al. A panel of nine cerebrospinal fluid biomarkers may identify patients with atypical parkinsonian syndromes. *Journal of neurology, neurosurgery, and psychiatry.* 2015;86:1240-1247
 114. Stewart T, Liu C, Gingham C, Cain KC, Auinger P, Cholerton B, et al. Cerebrospinal fluid alpha-synuclein predicts cognitive decline in parkinson disease progression in the datatop cohort. *The American journal of pathology.* 2014;184:966-975
 115. Park MJ, Cheon SM, Bae HR, Kim SH, Kim JW. Elevated levels of alpha-synuclein oligomer in the cerebrospinal fluid of drug-naïve patients with parkinson's disease. *J Clin Neurol.* 2011;7:215-222
 116. Tokuda T, Qureshi MM, Ardah MT, Varghese S, Shehab SA, Kasai T, et al. Detection of elevated levels of alpha-synuclein oligomers in csf from patients with parkinson disease. *Neurology.* 2010;75:1766-1772
 117. Hansson O, Hall S, Ohrfelt A, Zetterberg H, Blennow K, Minthon L, et al. Levels of cerebrospinal fluid alpha-synuclein oligomers are increased in parkinson's disease with dementia and dementia with lewy bodies compared to alzheimer's disease. *Alzheimer's research & therapy.* 2014;6:25
 118. Aasly JO, Johansen KK, Bronstad G, Waro BJ, Majbour NK, Varghese S, et al. Elevated levels of cerebrospinal fluid alpha-synuclein oligomers in healthy asymptomatic lrrk2 mutation carriers. *Front Aging Neurosci.* 2014;6:248
 119. Compta Y, Valente T, Saura J, Segura B, Iranzo A, Serradell M, et al. Correlates of cerebrospinal fluid levels of oligomeric- and total-alpha-synuclein in premotor,

- motor and dementia stages of parkinson's disease. *Journal of neurology*. 2015;262:294-306
120. Majbour NK, Vaikath NN, Eusebi P, Chiasserini D, Ardah M, Varghese S, et al. Longitudinal changes in csf alpha-synuclein species reflect parkinson's disease progression. *Movement disorders : official journal of the Movement Disorder Society*. 2016;31:1535-1542
 121. Wang Y, Shi M, Chung KA, Zabetian CP, Leverenz JB, Berg D, et al. Phosphorylated alpha-synuclein in parkinson's disease. *Sci Transl Med*. 2012;4:121ra120
 122. Stewart T, Sossi V, Aasly JO, Wszolek ZK, Uitti RJ, Hasegawa K, et al. Phosphorylated alpha-synuclein in parkinson's disease: Correlation depends on disease severity. *Acta Neuropathol Commun*. 2015;3:7
 123. Arendt T, Stieler JT, Holzer M. Tau and tauopathies. *Brain Res Bull*. 2016;126:238-292
 124. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between csf biomarkers and incipient alzheimer's disease in patients with mild cognitive impairment: A follow-up study. *The Lancet. Neurology*. 2006;5:228-234
 125. Zetterberg H. Cerebrospinal fluid biomarkers for alzheimer's disease: Current limitations and recent developments. *Curr Opin Psychiatry*. 2015;28:402-409
 126. Backstrom DC, Eriksson Domellof M, Linder J, Olsson B, Ohrfelt A, Trupp M, et al. Cerebrospinal fluid patterns and the risk of future dementia in early, incident parkinson disease. *JAMA Neurol*. 2015;72:1175-1182
 127. Alves G, Bronnick K, Aarsland D, Blennow K, Zetterberg H, Ballard C, et al. Csf amyloid-beta and tau proteins, and cognitive performance, in early and untreated parkinson's disease: The norwegian parkwest study. *Journal of neurology, neurosurgery, and psychiatry*. 2010;81:1080-1086
 128. Zhang J, Mattison HA, Liu C, Ghingina C, Auinger P, McDermott MP, et al. Longitudinal assessment of tau and amyloid beta in cerebrospinal fluid of parkinson disease. *Acta neuropathologica*. 2013;126:671-682
 129. Liu C, Cholerton B, Shi M, Ghingina C, Cain KC, Auinger P, et al. Csf tau and tau/abeta42 predict cognitive decline in parkinson's disease. *Parkinsonism & related disorders*. 2015;21:271-276
 130. Parnetti L, Tiraboschi P, Lanari A, Peducci M, Padiglioni C, D'Amore C, et al. Cerebrospinal fluid biomarkers in parkinson's disease with dementia and dementia with lewy bodies. *Biol Psychiatry*. 2008;64:850-855
 131. Compta Y, Marti MJ, Ibarretxe-Bilbao N, Junque C, Valldeoriola F, Munoz E, et al. Cerebrospinal tau, phospho-tau, and beta-amyloid and neuropsychological functions in parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 2009;24:2203-2210
 132. Mollenhauer B, Trenkwalder C, von Ahsen N, Bibl M, Steinacker P, Brechlin P, et al. Beta-amyloid 1-42 and tau-protein in cerebrospinal fluid of patients with parkinson's disease dementia. *Dementia and geriatric cognitive disorders*. 2006;22:200-208

133. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in alzheimer disease. *Nature reviews. Neurology*. 2010;6:131-144
134. Alves G, Lange J, Blennow K, Zetterberg H, Andreasson U, Forland MG, et al. Csf abeta42 predicts early-onset dementia in parkinson disease. *Neurology*. 2014;82:1784-1790
135. Siderowf A, Xie SX, Hurtig H, Weintraub D, Duda J, Chen-Plotkin A, et al. Csf amyloid {beta} 1-42 predicts cognitive decline in parkinson disease. *Neurology*. 2010;75:1055-1061
136. Alves G, Pedersen KF, Bloem BR, Blennow K, Zetterberg H, Borm GF, et al. Cerebrospinal fluid amyloid-beta and phenotypic heterogeneity in de novo parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry*. 2013;84:537-543
137. Lee MK, Cleveland DW. Neuronal intermediate filaments. *Annu Rev Neurosci*. 1996;19:187-217
138. Petzold A. Neurofilament phosphoforms: Surrogate markers for axonal injury, degeneration and loss. *Journal of the neurological sciences*. 2005;233:183-198
139. Neselius S, Brisby H, Theodorsson A, Blennow K, Zetterberg H, Marcusson J. Csf-biomarkers in olympic boxing: Diagnosis and effects of repetitive head trauma. *PloS one*. 2012;7:e33606
140. Hjalmarsson C, Bjerke M, Andersson B, Blennow K, Zetterberg H, Aberg ND, et al. Neuronal and glia-related biomarkers in cerebrospinal fluid of patients with acute ischemic stroke. *J Cent Nerv Syst Dis*. 2014;6:51-58
141. Gaiottino J, Norgren N, Dobson R, Topping J, Nissim A, Malaspina A, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. *PloS one*. 2013;8:e75091
142. Lu CH, Macdonald-Wallis C, Gray E, Pearce N, Petzold A, Norgren N, et al. Neurofilament light chain: A prognostic biomarker in amyotrophic lateral sclerosis. *Neurology*. 2015;84:2247-2257
143. Skillback T, Farahmand B, Bartlett JW, Rosen C, Mattsson N, Nagga K, et al. Csf neurofilament light differs in neurodegenerative diseases and predicts severity and survival. *Neurology*. 2014;83:1945-1953
144. Scherling CS, Hall T, Berisha F, Klepac K, Karydas A, Coppola G, et al. Cerebrospinal fluid neurofilament concentration reflects disease severity in frontotemporal degeneration. *Annals of neurology*. 2014;75:116-126
145. Abdo WF, Bloem BR, Van Geel WJ, Esselink RA, Verbeek MM. Csf neurofilament light chain and tau differentiate multiple system atrophy from parkinson's disease. *Neurobiology of aging*. 2007;28:742-747
146. Herbert MK, Aerts MB, Beenes M, Norgren N, Esselink RA, Bloem BR, et al. Csf neurofilament light chain but not flt3 ligand discriminates parkinsonian disorders. *Front Neurol*. 2015;6:91
147. Holmberg B, Johnels B, Ingvarsson P, Eriksson B, Rosengren L. Csf-neurofilament and levodopa tests combined with discriminant analysis may contribute to the differential diagnosis of parkinsonian syndromes. *Parkinsonism & related disorders*. 2001;8:23-31

148. Constantinescu R, Rosengren L, Johnels B, Zetterberg H, Holmberg B. Consecutive analyses of cerebrospinal fluid axonal and glial markers in parkinson's disease and atypical parkinsonian disorders. *Parkinsonism & related disorders*. 2010;16:142-145
149. Schultz NA, Johansen JS. Ykl-40-a protein in the field of translational medicine: A role as a biomarker in cancer patients? *Cancers (Basel)*. 2010;2:1453-1491
150. Bonneh-Barkay D, Wang G, Starkey A, Hamilton RL, Wiley CA. In vivo chi311 (ykl-40) expression in astrocytes in acute and chronic neurological diseases. *J Neuroinflammation*. 2010;7:34
151. Bonneh-Barkay D, Zagadailov P, Zou H, Niyonkuru C, Figley M, Starkey A, et al. Ykl-40 expression in traumatic brain injury: An initial analysis. *J Neurotrauma*. 2010;27:1215-1223
152. Bonneh-Barkay D, Bissel SJ, Kofler J, Starkey A, Wang G, Wiley CA. Astrocyte and macrophage regulation of ykl-40 expression and cellular response in neuroinflammation. *Brain pathology*. 2012;22:530-546
153. Bonneh-Barkay D, Bissel SJ, Wang G, Fish KN, Nicholl GC, Darko SW, et al. Ykl-40, a marker of simian immunodeficiency virus encephalitis, modulates the biological activity of basic fibroblast growth factor. *The American journal of pathology*. 2008;173:130-143
154. Olsson B, Lautner R, Andreasson U, Ohrfelt A, Portelius E, Bjerke M, et al. Csf and blood biomarkers for the diagnosis of alzheimer's disease: A systematic review and meta-analysis. *The Lancet. Neurology*. 2016;15:673-684
155. Wennstrom M, Surova Y, Hall S, Nilsson C, Minthon L, Hansson O, et al. The inflammatory marker ykl-40 is elevated in cerebrospinal fluid from patients with alzheimer's but not parkinson's disease or dementia with lewy bodies. *PloS one*. 2015;10:e0135458
156. Olsson B, Constantinescu R, Holmberg B, Andreassen N, Blennow K, Zetterberg H. The glial marker ykl-40 is decreased in synucleinopathies. *Movement disorders : official journal of the Movement Disorder Society*. 2013;28:1882-1885
157. Blum-Degen D, Muller T, Kuhn W, Gerlach M, Przuntek H, Riederer P. Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of alzheimer's and de novo parkinson's disease patients. *Neurosci Lett*. 1995;202:17-20
158. Muller T, Blum-Degen D, Przuntek H, Kuhn W. Interleukin-6 levels in cerebrospinal fluid inversely correlate to severity of parkinson's disease. *Acta Neurol Scand*. 1998;98:142-144
159. Yu SY, Zuo LJ, Wang F, Chen ZJ, Hu Y, Wang YJ, et al. Potential biomarkers relating pathological proteins, neuroinflammatory factors and free radicals in pd patients with cognitive impairment: A cross-sectional study. *BMC Neurol*. 2014;14:113
160. Lindqvist D, Hall S, Surova Y, Nielsen HM, Janelidze S, Brundin L, et al. Cerebrospinal fluid inflammatory markers in parkinson's disease--associations with depression, fatigue, and cognitive impairment. *Brain, behavior, and immunity*. 2013;33:183-189

161. Atik A, Stewart T, Zhang J. Alpha-synuclein as a biomarker for parkinson's disease. *Brain pathology*. 2016;26:410-418
162. Bjorkqvist M, Ohlsson M, Minthon L, Hansson O. Evaluation of a previously suggested plasma biomarker panel to identify alzheimer's disease. *PloS one*. 2012;7:e29868
163. Silajdzic E, Minthon L, Bjorkqvist M, Hansson O. No diagnostic value of plasma clusterin in alzheimer's disease. *PloS one*. 2012;7:e50237
164. Gisslen M, Price RW, Andreasson U, Norgren N, Nilsson S, Hagberg L, et al. Plasma concentration of the neurofilament light protein (nfl) is a biomarker of cns injury in hiv infection: A cross-sectional study. *EBioMedicine*. 2016;3:135-140
165. Rojas JC, Karydas A, Bang J, Tsai RM, Blennow K, Liman V, et al. Plasma neurofilament light chain predicts progression in progressive supranuclear palsy. *Ann Clin Transl Neurol*. 2016;3:216-225
166. Oertel W, Schulz JB. Current and experimental treatments of parkinson disease: A guide for neuroscientists. *Journal of neurochemistry*. 2016;139 Suppl 1:325-337
167. Fahn S ER. Updrs program members. Unified parkinson's disease rating scale. In: Fahn s, marsden cd, goldstein m, calne db, editors. Recent developments in parkinson's disease. Florham park, nj: Macmillan healthcare information;. 1987:153-163
168. Hoehn MM, Yahr MD. Parkinsonism: Onset, progression and mortality. *Neurology*. 1967;17:427-442
169. Morris S, Morris ME, Iansek R. Reliability of measurements obtained with the timed "up & go" test in people with parkinson disease. *Phys Ther*. 2001;81:810-818
170. Abdo WF, Borm GF, Munneke M, Verbeek MM, Esselink RA, Bloem BR. Ten steps to identify atypical parkinsonism. *Journal of neurology, neurosurgery, and psychiatry*. 2006;77:1367-1369
171. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12:189-198
172. Mohs RC, Cohen L. Alzheimer's disease assessment scale (adas). *Psychopharmacol Bull*. 1988;24:627-628
173. Andersson M, Wiig EH, Minthon L, Londos E. A quick test for cognitive speed: A measure of cognitive speed in dementia with lewy bodies. *American journal of Alzheimer's disease and other dementias*. 2007;22:313-318
174. Sebaldt R, Dalziel W, Massoud F, Tanguay A, Ward R, Thabane L, et al. Detection of cognitive impairment and dementia using the animal fluency test: The decide study. *The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques*. 2009;36:599-604
175. Isella V, Mapelli C, Siri C, De Gaspari D, Pezzoli G, Antonini A, et al. Validation and attempts of revision of the mds-recommended tests for the screening of parkinson's disease dementia. *Parkinsonism Relat Disord*. 2014;20:32-36
176. Shulman KI. Clock-drawing: Is it the ideal cognitive screening test? *International journal of geriatric psychiatry*. 2000;15:548-561

177. Gandek B, Ware JE, Aaronson NK, Apolone G, Bjorner JB, Brazier JE, et al. Cross-validation of item selection and scoring for the sf-12 health survey in nine countries: Results from the iqola project. International quality of life assessment. *Journal of clinical epidemiology*. 1998;51:1171-1178
178. Holland A, O'Connor RJ, Thompson AJ, Playford ED, Hobart JC. Talking the talk on walking the walk: A 12-item generic walking scale suitable for neurological conditions? *Journal of neurology*. 2006;253:1594-1602
179. Visser M, Marinus J, Stiggelbout AM, Van Hilten JJ. Assessment of autonomic dysfunction in parkinson's disease: The scopa-aut. *Movement disorders : official journal of the Movement Disorder Society*. 2004;19:1306-1312
180. Marinus J, Visser M, van Hilten JJ, Lammers GJ, Stiggelbout AM. Assessment of sleep and sleepiness in parkinson disease. *Sleep*. 2003;26:1049-1054
181. Cella D, Webster K. Linking outcomes management to quality-of-life measurement. *Oncology (Williston Park)*. 1997;11:232-235
182. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta psychiatrica Scandinavica*. 1983;67:361-370
183. Litvan I, Agid Y, Calne D, Campbell G, Dubois B, Duvoisin RC, et al. Clinical research criteria for the diagnosis of progressive supranuclear palsy (steele-richardson-olszewski syndrome): Report of the ninds-spasp international workshop. *Neurology*. 1996;47:1-9
184. Litvan I, Hauw JJ, Bartko JJ, Lantos PL, Daniel SE, Horoupian DS, et al. Validity and reliability of the preliminary ninds neuropathologic criteria for progressive supranuclear palsy and related disorders. *J Neuropathol Exp Neurol*. 1996;55:97-105
185. McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, et al. Diagnosis and management of dementia with lewy bodies: Third report of the dlb consortium. *Neurology*. 2005;65:1863-1872
186. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 2010;25:2649-2653
187. Association. AP. Diagnostic and statistical manual of mental disorders. 3rd ed, revised. Washington, dc: American psychiatric association. 1987
188. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of alzheimer's disease: Report of the nincds-adrda work group under the auspices of department of health and human services task force on alzheimer's disease. *Neurology*. 1984;34:939-944
189. Blom G. Statistical estimates and transformd beta-variables. New york: John wikey & sons. 1958
190. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. Mr signal abnormalities at 1.5 t in alzheimer's dementia and normal aging. *AJR Am J Roentgenol*. 1987;149:351-356
191. Schade S, Mollenhauer B. Biomarkers in biological fluids for dementia with lewy bodies. *Alzheimer's research & therapy*. 2014;6:72
192. International Parkinson Disease Genomics C, Nalls MA, Plagnol V, Hernandez DG, Sharma M, Sheerin UM, et al. Imputation of sequence variants for

- identification of genetic risks for parkinson's disease: A meta-analysis of genome-wide association studies. *Lancet*. 2011;377:641-649
193. Ishizawa T, Mattila P, Davies P, Wang D, Dickson DW. Colocalization of tau and alpha-synuclein epitopes in lewy bodies. *Journal of neuropathology and experimental neurology*. 2003;62:389-397
194. Moussaud S, Jones DR, Moussaud-Lamodièrè EL, Delenclos M, Ross OA, McLean PJ. Alpha-synuclein and tau: Teammates in neurodegeneration? *Mol Neurodegener*. 2014;9:43
195. Wills J, Jones J, Haggerty T, Duka V, Joyce JN, Sidhu A. Elevated tauopathy and alpha-synuclein pathology in postmortem parkinson's disease brains with and without dementia. *Exp Neurol*. 2010;225:210-218
196. Guo JL, Covell DJ, Daniels JP, Iba M, Stieber A, Zhang B, et al. Distinct alpha-synuclein strains differentially promote tau inclusions in neurons. *Cell*. 2013;154:103-117
197. Duka T, Duka V, Joyce JN, Sidhu A. Alpha-synuclein contributes to gsk-3beta-catalyzed tau phosphorylation in parkinson's disease models. *FASEB J*. 2009;23:2820-2830
198. Compta Y, Parkkinen L, O'Sullivan SS, Vandrovcova J, Holton JL, Collins C, et al. Lewy- and alzheimer-type pathologies in parkinson's disease dementia: Which is more important? *Brain : a journal of neurology*. 2011;134:1493-1505
199. Irwin DJ, White MT, Toledo JB, Xie SX, Robinson JL, Van Deerlin V, et al. Neuropathologic substrates of parkinson disease dementia. *Annals of neurology*. 2012;72:587-598
200. Strozzyk D, Blennow K, White LR, Launer LJ. Csf abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology*. 2003;60:652-656
201. Maetzler W, Liepelt I, Reimold M, Reischl G, Solbach C, Becker C, et al. Cortical pib binding in lewy body disease is associated with alzheimer-like characteristics. *Neurobiology of disease*. 2009;34:107-112
202. Palmqvist S, Mattsson N, Hansson O, Alzheimer's Disease Neuroimaging I. Cerebrospinal fluid analysis detects cerebral amyloid-beta accumulation earlier than positron emission tomography. *Brain : a journal of neurology*. 2016;139:1226-1236
203. Palmqvist S, Zetterberg H, Mattsson N, Johansson P, Alzheimer's Disease Neuroimaging I, Minthon L, et al. Detailed comparison of amyloid pet and csf biomarkers for identifying early alzheimer disease. *Neurology*. 2015;85:1240-1249
204. Janelidze S, Lindqvist D, Francardo V, Hall S, Zetterberg H, Blennow K, et al. Increased csf biomarkers of angiogenesis in parkinson disease. *Neurology*. 2015;85:1834-1842
205. Edison P, Ahmed I, Fan Z, Hinz R, Gelosa G, Ray Chaudhuri K, et al. Microglia, amyloid, and glucose metabolism in parkinson's disease with and without dementia. *Neuropsychopharmacology*. 2013;38:938-949

206. Fan Z, Aman Y, Ahmed I, Chetelat G, Landeau B, Ray Chaudhuri K, et al. Influence of microglial activation on neuronal function in alzheimer's and parkinson's disease dementia. *Alzheimers Dement.* 2015;11:608-621 e607
207. Levine J, Barak Y, Chengappa KN, Rapoport A, Rebey M, Barak V. Cerebrospinal cytokine levels in patients with acute depression. *Neuropsychobiology.* 1999;40:171-176
208. Stubner S, Schon T, Padberg F, Teipel SJ, Schwarz MJ, Haslinger A, et al. Interleukin-6 and the soluble il-6 receptor are decreased in cerebrospinal fluid of geriatric patients with major depression: No alteration of soluble gp130. *Neurosci Lett.* 1999;259:145-148
209. Bateman RJ, Wen G, Morris JC, Holtzman DM. Fluctuations of csf amyloid-beta levels: Implications for a diagnostic and therapeutic biomarker. *Neurology.* 2007;68:666-669
210. Bjerke M, Portelius E, Minthon L, Wallin A, Anckarsater H, Anckarsater R, et al. Confounding factors influencing amyloid beta concentration in cerebrospinal fluid. *Int J Alzheimers Dis.* 2010;2010
211. Slats D, Claassen JA, Spies PE, Borm G, Besse KT, van Aalst W, et al. Hourly variability of cerebrospinal fluid biomarkers in alzheimer's disease subjects and healthy older volunteers. *Neurobiology of aging.* 2012;33:831 e831-839
212. Spies PE, Slats D, Rikkert MG, Tseng J, Claassen JA, Verbeek MM. Csf alpha-synuclein concentrations do not fluctuate over hours and are not correlated to amyloid beta in humans. *Neurosci Lett.* 2011;504:336-338
213. del Campo M, Mollenhauer B, Bertolotto A, Engelborghs S, Hampel H, Simonsen AH, et al. Recommendations to standardize preanalytical confounding factors in alzheimer's and parkinson's disease cerebrospinal fluid biomarkers: An update. *Biomark Med.* 2012;6:419-430
214. Le Bastard N, De Deyn PP, Engelborghs S. Importance and impact of preanalytical variables on alzheimer disease biomarker concentrations in cerebrospinal fluid. *Clin Chem.* 2015;61:734-743
215. Mollenhauer B, Parnetti L, Rektorova I, Kramberger MG, Pikkarainen M, Schulz-Schaeffer WJ, et al. Biological confounders for the values of cerebrospinal fluid proteins in parkinson's disease and related disorders. *Journal of neurochemistry.* 2016;139 Suppl 1:290-317
216. Hall S, Surova Y, Ohrfelt A, Swedish Bio FS, Blennow K, Zetterberg H, et al. Longitudinal measurements of cerebrospinal fluid biomarkers in parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society.* 2016;31:898-905
217. Hall S, Ohrfelt A, Constantinescu R, Andreasson U, Surova Y, Bostrom F, et al. Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders. *Archives of neurology.* 2012;69:1445-1452
218. Hall S, Surova Y, Ohrfelt A, Zetterberg H, Lindqvist D, Hansson O. Csf biomarkers and clinical progression of parkinson disease. *Neurology.* 2015;84:57-63

Permissions to print

Paper I: Reproduced with permission from *Archives of Neurology (JAMA Neurology)* 2012. Volume 69 (No. 11): 1445-52. Copyright© (2012) American Medical Association. All rights reserved.

Paper II: Reproduced with permission from *Neurology* 84, no. 1 (2015): 57-63. Wolters Kluwer Health Lippincott Williams & Wilkins©.

Paper III: *Movement disorders*. 31, no. 6 (2016): 898-905. Open access.

Paper IV: Reproduced with permission from *Neurology*. Wolters Kluwer Health Lippincott Williams & Wilkins©.