Cerebrospinal fluid biomarkers in dementia with Lewy bodies

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2020

document version
Publisher's PDF, also known as Version of record

CHAPTER 8

Summary and General discussion

SUMMARY

The **general aim of this thesis** was to improve the (early) diagnosis of dementia with Lewy bodies (DLB) by developing cerebrospinal fluid (CSF) biomarkers.

Part 1: existing CSF biomarkers

In the first part of this thesis, we evaluated the potential of existing CSF biomarkers to improve accurate and timely diagnosis of DLB. The quantification of extracellular α-synuclein is probably the most obvious potential CSF biomarker for DLB, as it may reflect Lewy body pathology in the brain. The study described in **chapter 2** aimed to address this issue by investigating next to total α-synuclein, also the potential of the more disease-specific α-synuclein species - oligomeric α-synuclein and phosphorylated α-synuclein at Ser129 - as biomarkers for DLB. We found that CSF levels of total α-synuclein were lower in DLB and PD patients compared with both AD patients and controls, whereas CSF levels of oligomeric α-synuclein were higher in DLB and PD patients. Furthermore, we have shown that CSF α-synuclein species, in particular total α-synuclein and oligomeric α-synuclein, in combination with the established Alzheimer's disease (AD) biomarkers could be useful as a part of a biomarker panel to support DLB diagnosis.

Chapter 3 and **chapter 4** cover two studies on the established AD CSF biomarkers in DLB. In **chapter 3**, we described the prevalence of abnormal AD CSF biomarker levels, i.e. amyloid-β 1-42 (Aβ42), total Tau protein (t-tau) and Tau phosphorylated at threonine 181 (p-tau), across the spectrum of Lewy body diseases in a large multicenter cohort, including 375 DLB, 164 Parkinson's disease (PD) and 55 PD dementia patients from 10 centers. We showed that a substantial proportion of DLB patients have abnormal values for AD CSF biomarkers (Aβ42: 49%; t-tau: 37%; p-tau: 30%). Moreover, a CSF profile compatible with AD, defined as low Aβ42 in combination with elevated t-tau and p-tau, was more common in DLB (25%) compared to only 9% of PD dementia and 3% of PD patients. Our results suggest that CSF AD biomarkers cannot discriminate DLB from AD. To investigate whether AD pathology has clinical implications in DLB, we investigated in **chapter 4** the predictive value of CSF Aβ42, t-tau and p-tau on cognitive decline in DLB in a large sample of DLB patients who were followed longitudinally. We found that low baseline levels of Aβ42 alone or in combination with elevated Tau and p-tau were associated with a more rapid cognitive decline during 2-year-follow-up. Thus, the analysis of CSF AD biomarkers could help clinicians to better predict cognitive trajectories of individual DLB patients.

Chapter 5 focused on different isoforms of the amyloid-β peptide, in particular CSF Aβ38, Aβ40 and Aβ42 - the most abundant Aβ peptides in CSF. We showed a selective drop of CSF Aβ42 in AD, whereas in DLB lower levels of CSF Aβ42 were accompanied by lower overall Aβ peptide levels. Several other studies have also demonstrated evidence for lower CSF Aβ peptide levels in DLB, but we uniquely investigated CSF Aβ peptide levels relative to AD pathology. No differences in CSF Aβ40 and Aβ38 were found between DLB 'pure' patients and DLB patients with a CSF AD profile, suggesting that lower CSF Aβ40 and Aβ38 levels observed in DLB were independent of AD pathology. APOE ε4 genotype was related to neither CSF Aβ40 nor Aβ38 levels. Overall, the results of this study suggest disease-specific aberrations in Aβ peptide-related amyloid homeostasis in DLB versus AD.

Part 2: Novel CSF biomarkers

In the second part of this thesis, we accepted the challenge to discover novel biomarkers for DLB. In **chapter 6**, we applied state-of-the-art quantitative proteomic methods to assess CSF from a well-characterized cohort comprising DLB patients (*n* = 20) and age- and gender matched cognitively normal controls (*n* = 20). A total of 1995 proteins were identified, of which 69 were differentially expressed between DLB patients and controls. Independent cohort replication confirmed VGF, SCG2, NPTX2, NPTXR, PDYN and PCSK1N as candidate biomarkers for DLB. The identification of these six candidate biomarkers substantiate the importance of synaptic dysfunction in the pathophysiology of DLB. Furthermore, we showed that low levels of all biomarker candidates, except PCSK1N, were associated with cognitive decline. While levels of single proteins alone could not clearly discriminate between groups, random forest analyses using a panel of the identified biomarkers showed that the different levels of synaptic proteins enabled discrimination not only between DLB and controls, but also between DLB and patients with AD, PD and frontotemporal dementia (FTD). The putative CSF biomarkers identified in this study provide perspectives for future research.

Chapter 7 describes the methodological and clinical validation of VGF, one of the most promising novel biomarker candidates identified in our proteomic study, in a large and independent sample of patients with DLB, compared to both AD and cognitively normal controls. We used two different analytical methods: (1) in-house-developed quantitative competitive enzyme linked immunosorbent assays (ELISA), and (2) selected reaction monitoring (SRM). We showed that VGF levels in CSF were lower in DLB compared to both AD and controls across different analytical methods. In addition, we found that VGF levels were associated with the rate of decline in multiple cognitive domains. More specifically, lower VGF levels were associated with baseline cognition. In contrast, higher

VGF levels were associated with faster cognitive decline. The strong associations with cognitive decline point out that VGF could also be useful as a disease stage or prognostic biomarker for DLB.

GENERAL DISCUSSION

The main findings of this thesis are:

Part 1: existing CSF biomarkers

- CSF levels of total α-synuclein are lower in DLB and PD patients compared to AD patients and controls, whereas levels of oligomeric α-synuclein were higher in DLB and PD;
- CSF α-synuclein species, in combination with the established CSF AD biomarkers are useful as part of a biomarker panel to support DLB diagnosis;
- A substantial proportion of DLB patients have abnormal values for AD CSF biomarkers (Aβ42: 49%; t-tau: 37%; p-tau: 30%);
- A CSF profile compatible with AD is more common in DLB (25%) compared with PD dementia (9%) and PD (3%);
- Abnormal CSF AD biomarkers are associated with more rapid cognitive decline in DLB;
- CSF amyloid-β peptide profiles are different in DLB and AD: AD is characterized by an isolated drop in Aβ42, while DLB comes with reductions in Aβ38, Aβ40 and Aβ42.

Part 2: novel CSF biomarkers

- Using a state-to-the-art proteomic approach we identified VGF, SCG2, NPTX2, NPTXR, PDYN and PCSK1N as putative novel CSF biomarkers for DLB;
- The six identified biomarkers, particularly when used as a panel, show promise to improve diagnostic accuracy of DLB diagnosis (combination of VGF, SCG2 and PDYN differentiated between DLB and related neurodegenerative diseases with an accuracy > 80%);
- VGF levels in CSF were lower in patients with DLB compared to both AD patients and controls across different analytical methods;
- Lower VGF levels were associated with baseline cognition, whereas higher VGF levels were associated with faster cognitive decline.
- In the following paragraphs, these results are discussed in a broader context. Several methodological considerations and limitations of our work are provided.

Next, the clinical and therapeutical relevance of our findings are considered. We conclude with the future steps necessary to bring promising candidate biomarkers towards clinical practice.

Methodological considerations

Selection of study population

A strong aspect of this thesis is the use of clinically well-characterized cohorts. For the studies described in **chapter 2, 5-7**, we included patients selected from the Amsterdam Dementia Cohort.[1, 2] All patients and controls underwent an extensive and highly standardized clinical work-up at baseline and yearly follow-up. Diagnoses were made by consensus in a multidisciplinary meeting according to standard diagnostic criteria. Although histopathological confirmation is lacking for most cases, a major strength of this cohort is the availability of surrogate biomarkers to support etiological diagnosis. The diagnosis of DLB was supported by ^{123}I[FP-CIT] single photon emission computed tomography (DaT-SPECT) findings showing presynaptic dopaminergic deficits, or by slow-wave activity on EEG. By the use of these strict including criteria we ensure our clinical diagnoses were supported by the best pathophysiological evidence available, which is highly relevant for proof of concept and biomarker discovery studies. On the other hand, the use of selected patient populations might limit the external validity of our findings. Hence, the relevance of results from proof of concept or biomarker discovery studies will only become apparent in unselected validation cohorts, for which a large sample size is of utmost importance, especially for a complex disease as DLB. For the studies described in **chapter 3** and **chapter 4** we chose, therefore, a different approach. In these two studies, we investigated CSF AD biomarkers in a large retrospective multicenter cohort. This multicenter collaboration avoids several of the risks of biases associated with single center studies. However, limitations of this multicenter approach include heterogeneity due to differences in diagnostic processes and pre-analytical CSF procedures across centers, and the fact that CSF was analyzed using different analytical platforms. The differences between centers emphasize the importance of harmonization protocols and guidelines for pre-analytic sample handling, biochemical procedures as well as clinical assessment of patients.

The challenge of quantifying α-synuclein in CSF

Since the discovery of α-synuclein in extracellular biofluids in the early 2000,[3, 4] many research groups encountered difficulties in reliably detecting and quantifying α-synuclein species in biofluids (reviewed in [5-7]). As a result, the absolute concentration of α-synuclein in CSF varies largely across studies, due to variation in the nature of the assay and protein standards, differences in pre-analytical processing, blood

contamination from traumatic lumbar puncture - which have been shown to increase α-synuclein levels[8] – as well as variability in selection of patients and controls. In **chapter 2** we used in-house developed enzyme-linked immunosorbent assays (ELISA) with conformation-specific monoclonal antibodies that showed specificity towards α-synuclein aggregates. Immunohistochemical analysis showed that the monoclonal antibodies were able to recognize α-synuclein pathology in DLB. Quality parameters for these assays were acceptable.[9, 10] Nevertheless, the large variation with groups and overlapping values between groups raised the question whether our ELISA assay is a reliable method to detect and quantify Lewy body specific α-synuclein species in vivo. Clearly, further work is required to study CSF α-synuclein species in autopsy-verified cases and correlations with neuropathological findings. In addition, other methods to quantify α-synuclein in biofluids should also be explored.

The use of CSF biomarkers to capture concomitant AD pathology in DLB

Throughout this thesis we used several ways to define whether CSF biomarkers were indicative of concomitant AD pathology. In addition to including CSF AD biomarkers levels, i.e. Aβ42, t-tau and p-tau, as continuous variables, we used dichotomized values for individual biomarkers and the ratio of CSF t-tau/Aβ42. Assays for ante-mortem CSF measurements of Aβ42, t-tau and p-tau show direct associations with post-mortem severity of Aβ and tau pathology in DLB.[11-13] Several studies showed that the ratio of CSF t-tau/Aβ42 is the strongest surrogate measure for concomitant AD pathology in DLB.[13, 14] Currently, CSF p-tau/Aβ42 ratio is increasingly used in the clinic as surrogate marker for AD pathology, reflecting both increased tangle density and increased amyloid plaque deposition.[15] Larger clinicopathological studies should be performed to find out which ratio fits best for DLB patients. Instead of CSF measures we could also have used amyloid position emission tomography (PET) imaging as a proxy for concomitant AD pathology in DLB. Limitations for this approach are, however, the high costs, the involvement of radiation exposure and the limited availability of PET imaging.

Proteomics

The discovery of novel biomarkers is one of the most vibrant and important areas of research today. Proteomics is a promising technique for biomarker discovery, allowing for simultaneous measurement of large number of proteins present within a biological sample and to make data-driven, unbiased and hypothesis fee comparison of proteomes between healthy and diseases states. In **chapter 6** we used a state-of-the-art proteomic based approach to identify novel CSF biomarker candidates for DLB. Improvements in fractionation strategies and mass spectrometry platforms have increased the number of proteins that can be identified and quantified in a complex biological sample from

hundreds a decade ago to thousands today. Nevertheless, the complexity of the proteome results in a number of challenges. The proteomic pipeline is biased towards the identification of more abundant proteins. This is particularly a problem in mass spectrometry-based proteomic analysis of CSF, since most proteins secreted from the brain into the CSF (e.g. cytokines and neuropeptides) have low concentrations (< 100 pg/mL). For example, several known key pathological determinants of DLB, including α-synuclein and Aβ42, were not detected, since their CSF concentration are below the typical limit of detection of mass spectrometry methods. In addition, these proteins can be highly post-translationally modified, which further compromises mass spectrometry-based identification by default search strategies. Hence, the possibility cannot be excluded that we may have missed some potentially interesting proteins. Furthermore, a second hurdle in biomarker discovery using mass spectrometry methods is to go beyond the lists of candidate proteins and convert these candidate biomarkers into clinical applications. When identifying hundreds to thousands of proteins in few clinical samples, many biomarker candidates are expected to be false positives. Verification and validation experiments are necessary to test the large number of candidates in sufficiently large cohorts. For initial verification of our biomarkers candidates we used in **chapter 6** the same mass spectrometry workflow in an independent cohort and validated them using ELISA assays (**chapter 6** and **chapter 7**). Unfortunately, immunological analytical methods are limited by the dependence of high-quality and suitable antibodies, requiring expensive and time-consuming development of robust immunoassays and the low multiplexing potential. Therefore, we were only able to validate two out of six biomarker candidates using ELISA. Recently, target proteomic techniques (e.g. SRM) and antibody arrays that allow multiplex, high-throughput and sensitive biomarker validation, have emerged to bridge the gap between biomarker discovery and biomarker implementation.[16, 17]

Insights and implications

The work in this thesis has provided novel insights in the different types of pathology underlying DLB and implications that will facilitate the clinical implementation of CSF biomarkers.

CSF biomarkers of Lewy body pathology: not beneficial for the diagnosis of DLB yet

The quantification of α-synuclein in CSF has been proposed as a diagnostic biomarker for DLB, because of its central role in the pathophysiology of DLB.[18] Most studies (reviewed in [19-21]), including our study described in **chapter 2**, show decreased levels of total α-synuclein in CSF from patients with DLB compared to controls and AD patients.

Nevertheless, large variation in absolute levels of α-synuclein between studies, large within-group variability and overlapping values between diagnostic groups limit the clinical applicability of CSF α-synuclein as a single diagnostic biomarker for DLB. The different absolute concentrations of total α-synuclein among studies highlight the need for standardized analytical protocols and the generation of common reference materials to harmonize results. The large within-group variability makes interpretation of the levels for individual patients complicated. It has been hypothesized that a dual mechanism is possible underlying the changes in total α-synuclein levels in CSF.[6] In DLB patients, levels of total α-synuclein may be a competition between aggregation of α-synuclein into Lewy bodies (resulting in low CSF total α-synuclein levels) and release of the protein from degenerating synapse (resulting in high CSF total α-synuclein levels). Moreover, the overlapping values between diagnostic groups indicate that the current total α-synuclein ELISA assays are unable to accurately measure pathogenic α-synuclein. The detection of insoluble, presynaptic oligomers of α-synuclein has recently been proposed as a more sensitive and specific biomarker of α-synuclein pathology in DLB and PD. The approaches described by us and one other study[22] thus far show increased levels in DLB, but also large variability and overlap between DLB patients and controls. Therefore, current ELISA assays for oligomeric α-synuclein may have limited capability as a biomarker readout. Finally, phosphorylated α-synuclein has also limited diagnostic utility for DLB. We found no differences in CSF phosphorylated α-synuclein between any of the investigated diagnostic groups. Although this finding still needs to be replicated, the very low concentrations of phosphorylated α-synuclein in CSF limit reliable quantification of this α-synuclein specie with current analytical methods. Although single CSF α-synuclein ELISA measures does not seem to be reliable diagnostic markers for DLB, the first studies using Real-Time-Quaking-Induced Conversion (RT-QuIC) to detect pathogenic α-synuclein aggregates (oligomers and fibrils) are encouraging and showed remarkable diagnostic value in differentiating PD and DLB from non-synucleinopathies (AD and healthy individuals).[23, 24] For more details on this emerging technique to detect α-synuclein see Future directions.

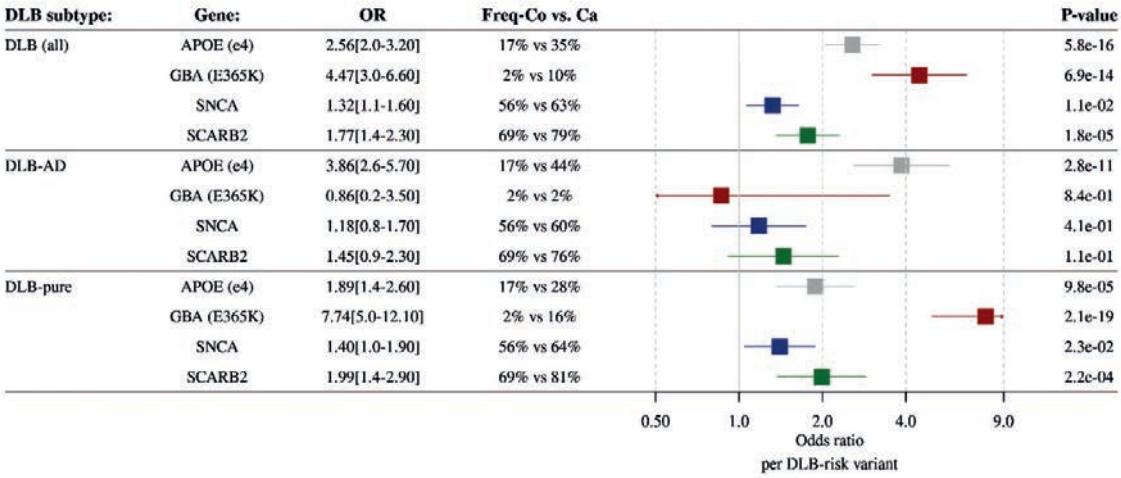
CSF biomarkers of AD pathology: can be abnormal and might be useful as prognostic markers in DLB

For physicians working in the dementia field, it is important to know that abnormal CSF AD biomarkers, i.e. Aβ42, t-tau and p-tau, does not exclude DLB in a differential diagnosis. In concordance with neuropathological studies, CSF studies consistently show that AD pathology is present in a significant number of DLB patients ante mortem (**Chapter 3**). Moreover, the majority of studies showed that CSF levels of Aβ42 in DLB are regularly decreased compared to cognitively normal controls (reviewed in [25, 26]) and

were not able to define valuable cutoff scores to distinguish DLB from AD.[27-29] Together, these results implicate that CSF AD biomarkers alone cannot discriminate between DLB and AD. However, our research demonstrates that AD pathology contributes to heterogeneity in rate of cognitive decline in DLB, CSF Aβ42 in particular is associated with more rapid cognitive decline over time (**Chapter 4**). In this line, other studies found that CSF AD biomarkers are linked to poor memory performance and earlier institutionalization and shorter survival in DLB.[30, 31] DLB patients with worse prognosis can currently be identified using CSF AD biomarkers, and we recommend that CSF AD biomarkers will be included in research categorization in DLB.

Although AD pathology is common in DLB, the factors contributing to the occurrence of AD pathology in DLB are still unclear. Age is an important risk factor for AD pathology,[32] and might also explain the differences in percentages of DLB cases with AD pathology between CSF studies and neuropathological studies.[33, 34] In addition, genetic factors may also contribute to the divergence in DLB patients who develop AD pathology.[34] In an ongoing study, we observed that the *APOE-ε4* allele was associated with an increased risk of an AD CSF profile in DLB, while for GBA the effect was opposite and was associated with an increased risk of DLB-pure (Figure 1). Furthermore, altered Aβ metabolism may also influence CSF Aβ42 levels in DLB. In **chapter 5** we found that while AD is characterized by an isolated drop in CSF Aβ42, DLB comes with reductions in Aβ38, Aβ40 and Aβ42. The mechanisms underlying the lower levels of all three Aβ peptides in DLB patients may be due to dysregulation in APP pathways as a consequence of reduced neuronal activity in DLB.[35, 36] Finally, animal and cellular models suggest synergistic interactions between α-synuclein pathology and AD pathology.[37, 38] Further research is necessary to elucidate how different pathologies evolve and interact to produce the heterogeneity in DLB.

Figure 1 | Association of DLB risk variants with DLB and DLB stratified by presence of co-existing AD pathology (van der Lee et al., submitted)



CSF biomarkers for synaptic dysfunction: non-specific for DLB but worthwhile to further investigate

Besides Lewy body pathology and AD pathology, more recent insights suggest a role for synaptic dysfunction in the pathogenesis of DLB.[39-41] Our findings of deregulated synaptic biomarkers in **chapter 6** and **chapter 7** highlight the importance of synaptic dysfunction in DLB and provide support to the link between synaptic dysfunction and cognitive decline in DLB. Although mechanisms remain to be elucidated, growing evidence indicates that synaptic dysfunction in DLB results from presynaptic deposition of toxic α-synuclein oligomers.[39-43] Our results indicate that VGF, SCG2, NPTX2, NPTXR, PDYN and PCSK1N are interesting markers of synaptic dysfunction in DLB. Although synaptic dysfunction is not specific for DLB, as there are also alterations of these markers in other neurodegenerative diseases, as for example AD,[44-48] we showed that the combination of VGF, SCG2 and PDYN differentiated between DLB and related neurodegenerative diseases with an accuracy > 80%. Our work strongly supports the further exploration of synaptic dysfunction in DLB, with potential to improve diagnosis and identify novel therapeutical targets.

Combination of CSF biomarkers: towards a biomarker-supported diagnosis of DLB

The complexity of the pathophysiological mechanisms underlying DLB, require, as the most reliable diagnostic approach, to measure a panel of biomarkers reflecting different aspects of the disease-related pathways. As specified throughout this thesis, the investigated CSF biomarkers cannot suffice as single biomarkers. Because of the considerable overlap in CSF levels between clinically related neurodegenerative

diseases, the ability of a single CSF biomarker to distinguish clinical dementia phenotypes on an individual patient level is limited. Alternatively, a panel of clearly defined biomarkers may provide a more robust and reproducible tool and may offer a more complete picture of disease and its underlying mechanisms. Indeed, the studies included in **chapter 2** and **chapter 6** of this thesis indicate that a combination of multiple biomarkers, reflecting the different aspects of the disease-related pathways of DLB, can reach remarkable diagnostic accuracies for DLB diagnosis (accuracy > 80%). Our results suggest that the biomarker panel for DLB should at least consist of the combination of CSF α-synuclein species, CSF AD biomarkers and CSF synaptic markers. We recognize that the proposed biomarker panel is not complete yet and can be extended with other yet to be determined biomarkers reflecting other biological mechanisms of DLB, including inflammatory markers (e.g. interleukins),[49, 50] lysosomal enzymes (e.g. GCase activity),[51, 52] lipid-binding proteins (e.g. fatty acid binding protein 3)[53] or brain neurotransmitters (e.g. acetylcholinesterase and dopamine.[54] More research to establish the optimal combination of biomarkers is warranted.

Future perspectives

The research in this thesis has contributed to a greater understanding of different types of pathology underlying DLB and reaching achievements with clinical potential for the diagnosis of DLB (Box 1). In particular, the introduction of a CSF biomarker panel that reflects the different aspects of the disease-related pathways in DLB, brings a biomarker-supported diagnosis of DLB a step closer. However, some issues remain and future research should endeavor to answer these.

Discovery of reliable diagnostic biomarkers for α-synuclein pathology

First, a reliable diagnostic biomarker for the signature pathology of DLB is still lacking. The seeding character of α-synuclein oligomers in a prion-like manner has sparked the idea that techniques originally developed for prion detection, such as real-time quaking induced conversion (RT-QuIC) or protein-misfolding cyclic amplification (PMCA), could be suitable for the detection of pathological forms of α-synuclein in CSF. The first studies using these techniques are encouraging. The application of RT-QuIC α-synuclein aggregates showed remarkable diagnostic value in differentiating PD and DLB from non-synucleinopathies (AD and healthy individuals).[23, 24] Two limitations of RT-QuIC are the presence of false negatives and the fact that the outcome is a binary measure, instead of a gradual scale. Nonetheless, the availability of α-synuclein aggregate assays will likely aid in the diagnosis of DLB and further studies using larger numbers and independent cohorts are necessary to assess the diagnostic utility of RT-QuIC α-synuclein.

Development and validation of trustworthy specific assays for synaptic markers

During the period in which this thesis has been written, novel techniques for measuring biomarkers have been developed rapidly, and techniques became more easily available, of which targeted proteomic techniques (e.g. SRM) show strong promise. The implementation of novel biomarkers is nowadays limited by the gap between discovery and validation. The emergence of targeted proteomics workflows has the potential for fast and cost-efficient verification of CSF biomarker candidates.[16, 17] In **chapter 7** we have provided a proof of principle for validation of one emerging synaptic biomarker using SRM. Further investigations into synaptic markers in DLB are warranted.

Translation of promising CSF biomarkers to blood and other biofluids

Recently, the biomarker field is undergoing a transition of focus from CSF towards other biofluids that can be obtained less invasively, such as blood. It would be meaningful to study the CSF biomarkers for DLB described in this thesis in other biofluids as well. Brain-derived proteins are, however, present at much lower concentrations in peripheral fluids than in CSF, which poses an analytical challenge. Although the research fields are still in its infancy, first results using novel ultrasensitive measurements techniques (e.g. SIMOA) are promising. For example, Aβ42 measured in blood using ultrasensitive measurement techniques can predict amyloid status with high accuracy.[55, 56] It is also worth to investigate other biofluids or peripheral tissues (e.g. saliva, tears, feces and skin). In this context, it has been shown that (1) changes in microbiome composition between PD and healthy controls have been consistently found (reviewed in [57]), (2) α-synuclein can be detected in saliva of PD patients,[58] (3) cutaneous α-synuclein in skin biopsy samples provides reasonable sensitivity and specificity for the detection of synucleinopathies (reviewed in [59]). Although numerous methodological challenges need to be addressed, including pre-analytical effects and statistical analysis methods for big data, the use of more easily accessible biofluids of peripheral tissues will facilitate serial sampling and allows for the investigation of changes in biomarker levels over time. This will likely provide novel insights in the emergence and progress of the different pathologies involved in DLB.

From a clinical towards a biological diagnosis

Early detection in the prodromal stage of the disease - the time at which emerging disease-modifying treatments may have the greatest effect – is of utmost importance. To achieve this goal a shift in the definition of DLB from syndromal to biological, in a manner similar as proposed in AD[15] is recommended. In other words, we should progress from characterizing DLB patients from a description of clinical symptomatology

towards a reliable and scalable biological characterization of pathology (i.e. from: 'this patient suffers from cognitive decline, visual hallucinations and parkinsonism' to 'this patient has a neurodegenerative disease caused by combination of both α-synuclein pathology and amyloid pathology'). A reliable α-synuclein biomarker is essential in that respect and could be of benefit to all synucleinopathies and the understanding of clinicopathological correlations. Moreover, information about underlying pathologies is crucial for the applicability of future disease modifying treatments as such therapies are likely to intercept early in the disease course before symptoms occur. CSF and other biofluid biomarkers may constitute an essential key towards a biological diagnosis antemortem. We envision that defining DLB as a biological construct will enable a more accurate characterization of the disease and promote efforts to understand how underlying pathologies evolve and interact. Furthermore, this approach will facilitate the identification of biological more homogenous patient populations most likely to respond to novel disease-modifying interventions that target the specific pathology, and thus open the way to personalized medicine. For example, DLB patients with evidence of the presence of AD pathology may benefit from AD-directed therapies. Future work integrating harmonized assessments of genetic factors, biochemical and imaging markers in patients followed until autopsy are required and will be critical to further refine the biological classification of DLB.

Concluding remarks

This thesis endorses the importance of CSF biomarkers in the diagnostic process of DLB. The complexity of the pathophysiological mechanism underlying DLB makes the development of a single, direct and specific biomarker for DLB complicated. Our findings indicate that a biomarker panel consisting of multiple biomarkers reflecting different aspects of the disease-related pathways can reach remarkable diagnostic accuracies for DLB diagnosis. We recommend that this panel at least includes biomarkers for α-synuclein pathology, AD pathology and synaptic dysfunction. Future studies in large longitudinal cohorts are necessary and are of tremendous value to arrive at timely and accurate diagnosis of DLB. Ultimately, biofluid biomarkers may constitute an essential key towards a 'tailor made' biological diagnosis for an individual DLB patient and with the emerge of disease-modifying treatments a biological diagnosis may facilitate personalized targeted treatment.

Box 1 | Current status of CSF biomarkers for dementia with Lewy bodies

- *CSF biomarkers of Lewy body pathology: not beneficial for the diagnosis of DLB yet*
  Although differences in CSF total α-synuclein and oligomeric α-synuclein have been observed in patients with Dementia with Lewy bodies compared with compared with controls and Alzheimer's disease patients (Chapter 2), overlap between diagnostic groups and large within-group variability limit the clinical utility of these markers.
- *CSF biomarkers of AD pathology: can be abnormal and might be useful as prognostic markers in DLB*
  Established Alzheimer's disease CSF biomarkers alone cannot discriminate between DLB and AD (Chapter 3), but can improve prognostic assessment, with CSF Aβ42 being a valid marker of cognitive decline (Chapter 4) and they might be useful in selecting DLB patients for future therapeutic trials of AD disease modifying treatments.
- *CSF biomarkers for synaptic dysfunction: non-specific for DLB but worthwhile to further investigate*
  Our findings of deregulated synaptic biomarkers (Chapter 6 and Chapter 7) highlight the importance of synaptic dysfunction in DLB and provide support to the link between synaptic dysfunction and cognitive decline.
- *Combination of CSF biomarkers: towards a biomarker-supported diagnosis of DLB*
  A combination of multiple CSF biomarkers reflecting different reflecting different aspects of the disease-related pathways of DLB, might enable more accurate diagnosis. A biomarker panel consisting of the combination of CSF α-synuclein species, CSF AD biomarkers and CSF synaptic markers is expected to reach the highest diagnostic accuracy (Chapter 2 and Chapter 6) and brings us a step closer towards a biomarker-supported diagnosis of DLB.

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