V. Conclusion

Neurodegenerative diseases are characterized by progressive nervous system dysfunction with a late-onset and a limited or no therapy options. Biomarkers are crucial to allow early disease as well as differential diagnosis. Within this thesis mass spectrometry (MS)-based methods for the quantification of the two established AD biomarker candidates, Aβ peptides and tau protein, is aimed to be developed and via differential proteome analysis novel potential biomarkers are identified in order to assess the CSF-based diagnosis for PD.

A. In this thesis for the first time the development of a novel direct infusion (DI)-MS based method including Aβ enrichment via immunoprecipitation (IP) for relative quantification of Aβ peptides is demonstrated. The results of DI-MS are compared to an established western blotting (WB) method for Aβ peptides analytics. Therefore, the ratios of three Aβ forms are calculated for WB and DI-MS showing consistent values across the different samples. The established Aβ DI-MS method includes the advantages from immune-based techniques (i.e. ELISA) where Aβ are specifically identified and the capability of multiplexing given by the targeted MS method (like HPLC-SRM). This enables a robust identification of multiple truncated/modified Aβ forms present in biological samples. In comparison to HPLC-MS analysis DI-MS furthermore presents a short analysis time, no analyte carryover and the possibility to use small volumes for the analysis. As outlook, more replicates should be produced to benchmark the method and perform antibody free sample enrichment (e.g. via solid phase extraction).

The second biomarker on focus in this thesis for AD research is the tau protein. Tau exists in six isoforms in adult human central nervous system and their relative abundance can be addressed in relation to AD. Hence, within this work an enrichment protocol via differential centrifugation is developed in order to identify several tau isoforms from cerebral cortex samples of AD and AgeCTRL individuals. Different tau isoforms are then identified by mean of two independent techniques, WB and nanoHPLC-MS. Peptides indicating isoform-specific tau regions are successfully identified via nanoHPLC-MS and they can be further quantified to address the implication of different
forms in AD. Moreover, since tau hyperphosphorylation is playing a key role in AD development, tau phosphorylation is investigated and three AD specific sites are identified. Finally, since tau should serve as biomarker in CSF, an IP-based enrichment of tau spiked in CSF matrix is established, showing identification of the protein from a concentration similar to endogenous levels.

B. Parkinson’s disease (PD) diagnosis can definitively be performed only post mortem and to allow early diagnosis novel biomarkers are needed. To identify potential biomarkers the proteome of CSF from de novo PD patients and healthy control (CTRL) individuals are compared within a differential MS-based study in this thesis. To accomplish this task a label-free MS-based proteomic workflow is firstly optimized and characterization of CSF is performed in regards of its proteomic composition. CSF biomarker studies usually results in hundreds of potential biomarkers which might not be validated in a next step. Several factors can influence the outcome of a study (e.g. sample preparation, study design, data analysis).

The aim in this project is to establish an optimized workflow which leads to a robust CSF analysis. Therefore two strategies are implemented, a machine learning approach in which two discovery sets are compared and a combination of two quantification software within one discovery set. With the first strategy it is possible to perform a comprehensive analysis including biomarker identification and validation. This approach shows that a label-free CSF analysis at two different time points results in high variation and could not be used for validation, highlighting the importance of careful study design. An alternative approach considers the combination of two software for the selection of potential candidates in order to be stricter and to exclude false positive. With this strategy 13 putative candidates are identified and they show a sensitivity of 79 % and specificity of 77 % in discriminating PD from CTRL. One of the proteins selected by our method has already confirmed to be involved in PD as reported in a parallel study performed on the same samples with a CSF metabolomics platform.

Furthermore, two proteins among the 13 could be validated in an independent study group. In addition to the baseline biomarker discovery a longitudinal study is performed with CSF samples obtained from three different time points of the same PD and CTRL individuals. This is the first reported longitudinal biomarker study for PD in CSF.
Three protein candidates from the baseline discovery phase are confirmed in the PD follow-up study suggesting these proteins as suitable biomarker candidates. Summarized, the identified protein biomarker candidate signature could support PD diagnosis; some candidates already show positive results in an independent cohort as well as in a longitudinal study.