Summary and general discussion

Box 1. Main recommendations on (pre-)analytical factors in CSF to optimise biomarker studies resulting from this thesis.

Recommendations for pre-analytical processing factors in CSF:

- Maximum two hours delay between CSF withdrawal and centrifugation can be safely applied.
- Maximum two hours delay between CSF centrifugation and -80°C storage can be safely applied.
- Tube transfers of CSF should be avoided, especially for \(\alpha_b\), or the \(\alpha_b/a\beta\) ratio should be used to correct for \(\alpha_b\) loss due to adsorption.

Recommendations for (pre-)analytical factors in CSF biomarker studies:

- Freeze/thaw cycles (tested up to 7) can be safely applied to the majority of CSF proteins tested, but should always be tested for novel biomarkers or novel methods.
- Long-term biobank storage at -80°C can be safely applied for the biomarkers \(\alpha_b\), t-tau, p-tau, at least up to 12 years of storage.
- Evaporation does not occur in biobanking tubes stored at -20°C or -80°C, at least up to 4.5 years of storage.
- An unbiased statistical approach can correct for the upward assay drift of \(\alpha_b\).
- Assay comparison studies help to translate results from the same biomarker measured using different assays, which facilitates further biomarker validation steps.
- The automated Elecsys \(\alpha_b\), t-tau, and p-tau assays can be a good replacement for the manual Innotest \(\alpha_b\), t-tau, and p-tau assays.

Summary of the findings

In chapter 1 we delineated the context for this thesis by introducing international consensus guidelines for collection and storage procedures for CSF biobanks, thereby highlighting the issues for which more evidence is needed to support these guidelines. We further explained how biomarkers in CSF are essential for diagnosis and treatment development in AD, but that variability in CSF biomarker results hampers interpretation of these results. We therefore set out to systematically test pre-analytical and analytical confounders of CSF biomarker studies to increase the reproducibility of CSF biomarker studies in the subsequent chapters. In chapter 2 we showed that evaporation does not occur during biobank storage at -20°C or -80°C for the (body) fluids CSF, plasma, serum, saliva, or water, for up to 4.5 years. This finding supports the use of historically collected patient cohorts stored in biobanks, as long-term storage is not compromised by evaporation of the samples. In chapter 3 we concluded that the CSF...
biomarker concentrations for αβ_{1-42}, t-tau, and p-tau did not change upon -80°C biobank storage for up to 12 years. Our study design assumed that the mean biomarker concentrations in an homogeneous AD patient cohort would remain constant on group level over time, thereby allowing measurement of the biomarkers in patient samples with different storage times (2 – 14 years) while excluding assay batch interference. Although biomarker concentrations, measured using one assay batch, remained stable regardless of the biobank storage time, the originally reported αβ_{1-42} concentrations of the historical samples revealed that the αβ_{1-42} assay shows an upward drift over the years. This finding confirms that historical samples remain appropriate for biomarker studies, however, historically measured αβ_{1-42} results should be corrected for their year of measurement or should be remeasured. In chapter 8, we demonstrated that concentrations of the historical samples revealed that the αβ_{1-42} assay shows an upward drift over the years. This finding confirms that historical samples remain appropriate for biomarker studies, however, historically measured αβ_{1-42} results should be corrected for their year of measurement or should be remeasured. In chapter 8, we demonstrated that concentrations of the historical samples revealed that the αβ_{1-42} assay shows an upward drift over the years. This finding confirms that historical samples remain appropriate for biomarker studies, however, historically measured αβ_{1-42} results should be corrected for their year of measurement or should be remeasured. In chapter 4, we developed an unbiased, data-driven statistical model to correct the CSF αβ_{1-42} biomarker results from our Amsterdam Dementia Cohort (4397 patient samples) for the upward drift in CSF αβ_{1-42} concentrations over the last two decades. We verified the statistically corrected concentrations with the remeasured concentrations in a sub-cohort. This model for drift corrections is a useful tool to standardize CSF αβ_{1-42} biomarker data over time and amongst different centres or platforms.

In the next chapters, we zoomed in on the effects of pre-analytical processing steps on the CSF biomarker concentrations. In chapter 5, we found that CSF αβ_{1-42} concentrations were reduced during tube transfers of CSF, with a 5% decrease on average per transfer, of which half of the αβ_{1-42} concentration decreased due to pipetting only. We recommended to restrict the number of transfers during CSF work-up and to use the ratio αβ_{1-42}/αβ_{1-40} as this ratio was not affected by the variation caused by adsorption to lab plastics. In chapter 6 we tested the stability of 11 novel CSF biomarker candidates under standardised pre-analytical conditions that included storage at room temperature or 4°C up to one week, or at -20°C up to 1 month, or up to 7 freeze/thaw cycles. One metabolite, MHPG, which is the principal metabolite of the neurotransmitter norepinephrine, showed a strong and linear decrease in concentration upon storage conditions and after f/t cycles. However, the other biomarkers did not change under these conditions, suggesting that these biomarkers can be trustfully tested under the pre-analytical conditions present across different cohorts. In chapter 7 we extended this analysis to a larger range of proteins. Two array-based biomarker discovery platforms were used, SOMAscan and Olink, detecting both about a thousand proteins per sample. We defined stability criteria for the individual proteins based on the confidence interval of the difference between two extreme storage of freeze/thaw conditions. We found that >70% of the proteins remained stable under the most extreme conditions of one week storage at room temperature or after 7 freeze/thaw cycles compared to the reference sample. This indicates that the large majority of CSF proteins remains stable when exposed to time delays between processing steps up to one week, or multiple freeze/thaw cycles. In chapter 8 we demonstrated that concentrations...
of the potential biomarker progranulin remained stable in serum and CSF when samples were exposed to standardised pre-analytical conditions. Additionally, we showed that progranulin levels measured with two different commercial immunoassays correlated well, indicating that this biomarker can be further validated in clinical samples. In chapter 9 we compared three different immunoassays for the potential CSF biomarker neurogranin. We tested their epitope affinities and compared the absolute neurogranin levels in the same CSF samples of AD, FTD, VaD, DLB patients and control subjects. Although the absolute concentration ranges differed amongst the assays, the discriminative power of the assays amongst the clinical groups was comparable. The AD group presented with the highest neurogranin levels, but we found limited power for neurogranin as a differential diagnosis biomarker for dementia. In chapter 10 we found high concordance in CSF biomarker results measured with the automated Elecsys assays and the routine manual Innotest assays for Aβ1-42, t-tau and p-tau. CSF samples had been prospectively collected in local memory clinics in the Netherlands and thus closely reflect a real-life diagnostic setting. Results support the transition from the manual to the automated assays for the AD CSF biomarkers for clinical routine in research setting.

In this thesis we deciphered crucial factors of pre-analytical and analytical variation to increase awareness on how these factors influence the reproducibility of CSF testing, as well as how to manage these variation factors in order to increase reproducibility (Box 1). For CSF processing, we found that time delay between processing steps was not influencing biomarker concentrations, but that tube transfers of CSF should in principal be avoided. As for the pre-analytical factors in CSF biomarker studies, we found that long-term storage (up to 12 years) did not affect the AD biomarkers, nor did additional freeze/thaw cycles (up to 7), although storage and freeze/thaw stability testing should be applied to novel biomarkers and novel methods. Furthermore, we showed that the upward assay drift of Aβ1-42 over the last years can be statistically corrected for. For routine CSF AD biomarker measurements, we found that the recently developed fully automated Elecsys assays showed high concordance with the manual Innotest assays, thereby supporting the transition from manual to automated tests. Direct assay comparison studies for CSF biomarkers presented in this thesis helped the translation of results amongst the different assays, which facilitates further development of the biomarkers towards clinical implementation. Overall, increased reproducibility of CSF biomarker testing will support the clinical implementation of AD diagnostic biomarkers and facilitates the development of novel biomarkers for AD and other neurodegenerative diseases.

**Recommendations for CSF stability testing**

Next to the recommendations for increased reproducibility of CSF biomarker studies (Box 1), several tools have been developed in the course of this thesis. These applications were partly...