CHAPTER 9

Summary and general discussion

‘Too many pieces of music finish too long after the end’

Igor Stravinsky
General objective

After decades of research, we now reached a time in which CSF biomarkers for AD, Aβ42, tau and p-tau, are available and even included as evidence for underlying AD pathology in the diagnostic criteria. Clinicians increasingly use these markers, also in smaller local hospitals. However, these established CSF biomarkers perform suboptimal in early stages of Alzheimer’s disease, and do not reflect severity of the disease. This may be due to the heterogeneous nature of AD, and other pathological processes, beyond amyloid pathology, which we are only beginning to understand.

The aim of the first part of this thesis was to explore how the established CSF biomarkers Aβ42, tau and p-tau are currently used in clinical practice, and how they should best be used and combined in the memory clinic routine. The second part of this thesis was dedicated to investigating novel biomarkers for mechanisms causing heterogeneity in AD and for processes beyond amyloid pathology, that may be useful for early diagnosis.

PART 1: Clinical impact of CSF biomarkers and current practice

1.1 Summary of the findings

In chapter 2 we investigated which of the previously proposed combinations of biomarkers (consisting of Aβ42, tau and/or p-tau) constitutes the optimal ‘CSF AD profile’ to be used in clinical practice. We constructed eight AD profiles based on previously published combinations, including regression formulas and simple ratios. We compared their diagnostic accuracy and ability to predict dementia due to AD in 1385 patients from the Amsterdam Dementia Cohort, and the results were validated in an independent cohort from a large multicenter study (n=1442). We found that 1) every combination of biomarkers performed better than individual biomarkers for diagnosing and predicting AD, and 2) any ratio, either simple ratios or regression formulas, performed better than combinations based on dichotomized individual biomarkers, but 3) results were remarkably similar between the different ratios. The results were very similar in the validation cohort. Most importantly, the simple ratio tau/Aβ42, with a cut-off value of 0.52 (higher being abnormal), showed substantial robustness and validity.

In chapter 3 we investigated the impact of CSF AD biomarkers on clinical decision-making in the daily practice of a tertiary memory clinic. We included all patients (n=438) visiting the VUMc Alzheimer center for cognitive screening during one year, of which 351 (80%) underwent LP. Neurologists completed questionnaires before and after CSF disclosure. We assessed change of diagnosis, diagnostic confidence, and impact on patient management. We found that after disclosure of CSF 23/351 diagnoses (7%) were changed; among these, eight were
patients with an unclear pre-CSF diagnosis, who received a diagnosis after biomarker disclosure. Diagnostic confidence increased from 84% to 89%. There were consequences for management in 44/351 patients (13%) with CSF, and 13/87 patients (15%) because of unavailable CSF. This suggests that CSF biomarkers have additional value on top of the standard diagnostic work-up of cognitive disorders, especially in the subgroup of patients with an unclear or uncertain clinical diagnosis. Finally, we retrospectively applied the recently published ‘appropriate use criteria’ for amyloid-imaging (using PET) to our cohort. We found that amyloid-imaging would have been indicated for more than 40% of the patients, and that this proportion showed reasonable but not complete overlap with the clinician’s wish to use biomarkers. Most importantly, clinicians more often indicated they wished to use CSF results to rule out AD, while criteria for PET were not met.

In the study described in chapter 4 we investigated in detail the invasiveness and patient-burden of the LP procedure itself, in a multicenter setting (23 centers, in total 3868 patients). Prior to LP various patient characteristics and details on the LP procedure were recorded. Within two weeks after LP patients were asked about their complaints. We assessed the patient acceptance rate, and the frequency of and risk factors for post-LP complaints. Logistic regression analysis using generalized estimated equations was used to investigate factors associated with post-LP complications. We found that acceptance rate of LP was high (92%), even when the LP was performed for research purposes only (86%). Complaints of any kind occurred quite frequently (31%), but typical post-LP headache (PLPH) was present in only 9% of the patients, and complications needing medical intervention were very rare (1%). Fear for complications was an important risk factor for actually experiencing post-LP complaints, except for typical PLPH. Furthermore, patient characteristics, especially history of headache and younger age, seemed equally important risk factors for PLPH as LP-procedure characteristics such as a cutting-edge needle type and larger needle diameter. Patient-related risk factors for local back pain were similar to those for headache, while the only important procedure-related risk factor for back pain was number of LP attempts.

1.2 Discussion of the findings

Is there an optimal CSF AD profile?

Despite all the effort that has been put in development and validation of CSF Aβ42, tau and p-tau, it is still difficult to establish cut-off values for these biomarkers in such a way that they are generally applicable. Biomarker values are known to show substantial inter-site variability due to various pre-analytical, analytical and post-analytical factors. Moreover, there is discussion in the field whether either the cut-off levels of individual biomarkers or the ratio between Aβ42 and (p-)tau would be most informative. In the diagnostic framework of the International Working Group (IWG), biomarkers are mentioned without further specification; evidence of Alzheimer pathology includes both amyloid and tau. A recent update of the IWG
criteria, published after completion of our study, specifies this further; now both Aβ42 and (p-)tau should be abnormal in CSF to count as evidence for Alzheimer pathology. The National Institute on Aging-Alzheimer Association (NIA-AA) guidelines, published one year after the first IWG criteria, are even more detailed. In these guidelines biomarkers (also including nuclear imaging and MRI) are categorized according to presumed pathophysiological substrate and considered to reflect separate processes. But here ‘conflicting’ biomarkers (e.g. abnormal amyloid markers in combination with normal injury markers or vice versa) are assumed to be ‘uninformative’. To test whether these assumptions hold truth in clinical practice, we assessed combinations of individually dichotomized biomarkers, in addition to simple ratios of (p-)tau to Aβ42 and previously published regression formulas – i.e. complex, weighted ratios of biomarkers. Our conclusion that all ratios were more accurate than combinations of dichotomized biomarkers was mainly based on a higher sensitivity and negative predictive value of the ratios, with similar specificity. This suggests that a ratio of two markers is more sensitive, and that ‘conflicting’ biomarkers are not necessarily ‘uninformative’. This may be due to variations in (pre- or post-) analytical procedures, leading to variations in the protein content of CSF. A ratio of tau to Aβ42 could seemingly reduce these problems, providing more sensitive information about the presence of Alzheimer pathology, especially at borderline values.

The regression formulas we evaluated in this study can be seen as complex ratios with a different slope and intercept as simple ratios, based on specific and highly selected research populations. We hypothesized that this would likely hamper generalizability. Nevertheless, test characteristics of the different regression formulas were all very similar to each other, showing that a formula developed in one laboratory can easily be applied in a different setting. In addition, the simple ratio of tau/Aβ42 performed as good as complex regression formulas, in our cohort as well as in the independent multicenter cohort, while the cut-off of >0.52 was derived from our single center cohort. We concluded therefore that, despite inter-site and inter-assay variations, performance of these different ratios was robust. An explanation may be that the prevalence of borderline AD profiles is relatively low, and as such the regression formulas have a preciseness that does not increase diagnostic accuracy measures in a clinical setting. Hence, our study shows that there is not one optimal CSF AD profile, but several equally performing profiles, provided they are based on a ratio of tau and Aβ42. Based on the principle of parsimony however, the simple ratio of tau to Aβ42 is preferential over complex regression formulas. Clinicians should be able to calculate it easily, and the cut-off value of more than 0.52 being abnormal gives a useful rule of thumb: an AD profile in CSF is present whenever tau is more than half the value of Aβ42.

When do we need CSF biomarkers?

CSF biomarkers have been included in the diagnostic criteria for AD, without any specific guideline on how and when to use them. It is therefore important to know how clinicians use
this information in their clinical routine, as a starting point for guidelines on how they should use it. ‘Criteria for appropriate use’ have recently been published for amyloid imaging by use of PET, which is also included as biomarker in the diagnostic criteria. For CSF biomarkers however, there are no such criteria available.

The study described in chapter 3 has two main conclusions. First, CSF biomarkers have a confirmative role in most cases, leading to a modest increase of diagnostic confidence in cases where clinicians beforehand expressed the wish to use biomarkers and diagnostic confidence was lower than average. On the other hand, one third of the patients with an unclear pre-CSF diagnosis received a diagnosis after disclosure of CSF results, and there were consequences for management in a substantial proportion of patients, such as more intensive follow-up, or referral to another specialist. This proportion was similar in patients who had not undergone LP, meaning that if no CSF was available there were consequences for management in the same extent as when CSF was available.

Our finding that 7% of diagnoses changed due to CSF biomarker results is in contrast to previous studies performed on this subject, as the proportion is considerably higher in previous studies on the impact of either CSF biomarkers (up to 27%) or amyloid-PET imaging (up to 55%). In these previous studies however, there was generally a much lower pre-test diagnostic confidence compared to this study. This was either due to the inclusion criteria – some studies only included patients with an uncertain diagnosis – or due to the fact that the study was retrospective. It may be expected that in routine diagnostic practice ancillary investigations such as amyloid-PET or CSF biomarkers are performed only in patients with uncertain diagnoses. In this way, the patients not needing these investigations are not included in the considerations, while characterizing this group is also important for development of guidelines on appropriate use of biomarkers. In our center CSF biomarkers are performed in all patients as part of the general research protocol (provided they give informed consent), but the results are not part of the initial clinical decision-making. As such, we could perform this study prospectively, and we could include all patients presenting at our memory clinic to avoid potential inclusion bias. Clinical impact may not be as large as suggested by previous studies, but our study probably gives a more realistic view of the daily practice in a memory clinic. Finally, as the ‘appropriate use criteria’ for amyloid PET-imaging did not show complete overlap with the wish to use CSF biomarkers, we concluded that these criteria cannot be simply applied to CSF biomarkers.

**Patient burden of lumbar puncture**

An important issue currently hampering widespread use of CSF biomarkers is fear for complications of the lumbar puncture. It is not known to what extent fear for post-LP complications is justified, as the prevalence mentioned in previous literature varies widely. Occurrence of post-LP complications probably depends on numerous factors such as age.
and gender of the patients, needle characteristics and whether the LP is performed for spinal anaesthesia or in a diagnostic setting. Most previous studies were however either performed in relatively small patient samples, or in a randomized trial setting, not allowing for simultaneous investigation of all possible risk factors. Moreover, they were performed in other, mostly younger, populations. Prevalence of complaints and risk factors may be different in an elderly memory clinic population.

We showed in our study on LP feasibility in chapter 4 that the patient acceptance rate was high, suggesting that this fear is not as decisive as critics might assume. Furthermore, we found that although 3 out of 10 patients reported complaints in general, less than 10% reported typical post-LP headache. The few studies on post-LP complications that had been done previously in memory clinic populations all reported substantially lower complication rates, of even less than 1%. However, these studies may have underestimated the prevalence, as PLPH was either only registered when patients reported these complaints spontaneously, or after a single contact by a nurse the morning following LP. Hence, many patients may have had subclinical complaints, or were not yet experiencing any complaints at follow-up, as it is known that in up to one third of patients PLPH starts after 48 hours.

Our approach was different, as we actively asked patients whether they had experienced post-LP complaints, and in addition asked specific questions on severity and duration of the headache and back pain. Therefore, our study gives a more comprehensive and detailed overview, although the 1% of patients with complications serious enough to require medical intervention is probably most relevant in this respect. In addition, future studies should assess patient discomfort before, during and after procedures for other biomarker modalities than CSF analysis, as patients undergoing for example MRI or PET scans may experience anxiety, claustrophobia or other complaints of which the incidence is unknown. Hence it is currently not possible to compare patient experience between these procedures.

Regarding risk factors for PLPH, we confirmed previous results that the amount of CSF taken as well as bed rest after LP does not influence the incidence of PLPH, but younger patients had an increased risk for developing PLPH. As there was a lower incidence of back pain and non-specific headache with increasing age as well, it could be argued that this age effect is a more general phenomenon of decreasing pain sensitivity in elderly individuals. Besides the effect of age, we found that a diagnosis of MCI or dementia was associated with a lower risk of post-LP complaints compared to a non-neurodegenerative diagnosis. This had not been demonstrated so clearly before. It may either be due to short-term memory problems of these patients and therefore a reporting bias, or might be related to brain atrophy with increased CSF volume, although this has not yet been demonstrated experimentally. On the other hand, we found no gender difference, whereas PLPH has repeatedly been reported to be more common in women than men. Since especially women under the age of 40 tend to have a substantially higher risk of PLPH, it is plausible that gender effects disappear with
increasing age, and is negligible in an average memory clinic population. It is also important to note that fear for complications was an important risk factor for actually experiencing post-LP complaints, except for typical PLPH. Hence, there may be psychological factors, such as personality traits, relating to more complaints.

Finally, the needle characteristics were not as important as we had hypothesized. Needle type, but not diameter, was associated with typical PLPH; needle diameter was only associated with severe headache. However, our hypothesis (and the general consensus) on the importance of needle characteristics was based on case-control studies, only investigating a specific needle characteristic (i.e. either size or type). There were no previous studies simultaneously investigating needle characteristics and patient characteristics. We showed that, when all factors are included, factors related to patient characteristics are at least as important for post-LP complaints as LP-procedure characteristics.

PART 2: Heterogeneity of AD and new perspectives beyond amyloid

2.1 Summary of the findings

Chapter 5 and chapter 6 cover two studies on the Apolipoprotein-E ε4 (APOE4) allele and cognitive decline. In chapter 5 we investigated predictive value of APOE4 and CSF biomarkers for progression to MCI or dementia due to AD in patients with subjective cognitive decline. Cox proportional hazard models were used to assess individual and combined predictive value of APOE genotype and NIA-AA preclinical AD stages based on CSF biomarker concentrations (0: normal biomarkers; 1 abnormal Aβ42 only; 2: both abnormal Aβ42 and tau and/or p-tau; SNAP: Suspected Non-AD Pathology, indicated by abnormal tau and/or p-tau only). Patients showing clinical progression were more often APOE4 positive (63% vs. 38%, p<0.05) and their CSF biomarker concentrations more often indicated preclinical AD. We found that both APOE4 carriership and CSF biomarkers were associated with an increased risk of clinical progression. CSF biomarkers increased the risk of clinical progression in a dose dependent manner; isolated abnormal Aβ42 gave a less elevated risk compared to abnormal levels of both Aβ42 and (p-)tau. When APOE4 status and biomarker abnormalities were entered into a combined model, we found an interaction between the two. Therefore, we estimated predictive value of biomarker abnormalities for APOE4 carriers and non-carriers separately. This analysis showed that in APOE4 non-carriers CSF biomarker abnormalities strongly increased risk of clinical progression in a dose-dependent manner. In contrast, in APOE4 carriers there was only an increased risk of clinical progression when both Aβ42 and (p-)tau were abnormal, but not when only Aβ42 was abnormal. When comparing the subgroup of APOE4 carriers and non-carriers without any biomarker abnormalities, there was a trend towards a higher risk of cognitive decline in the APOE4 carriers.
In chapter 6 we investigated the influence of APOE4 on the association of change in CSF F$_2$-isoprostanes, a marker for oxidative stress, with cognitive decline. Twenty non-demented subjects, 58 MCI patients and 63 AD patients with measurements of CSF F$_2$-isoprostanes at two time points and known APOE genotype were included. For change in F$_2$-isoprostanes over time and associations with change in MMSE age- and sex-adjusted linear mixed models were used. Our group had shown before that increase of F$_2$-isoprostanes was similar across the different diagnostic groups. In the current study we found a larger increase over time in CSF levels of F$_2$-isoprostanes in APOE4 carriers than in non-carriers. Moreover, change in CSF F$_2$-isoprostanes was related to cognitive decline in APOE4 carriers, but not in non-carriers. These results suggest that oxidative stress is relatively more important in APOE4 carriers than in non-carriers in the neuropathological cascade leading to cognitive decline in AD.

In chapter 7 we explored the possible involvement of the blood-brain barrier (BBB) in AD. Previous studies are inconsistent on whether there is increased BBB permeability in AD. However, decreased BBB integrity has been implicated in cerebral amyloid angiopathy (CAA), which is in turn present in a large proportion of AD patients. We investigated whether matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs), proteins involved in BBB breakdown, were altered in CSF and plasma of AD patients, and whether this effect was modified by presence of microbleeds. We measured MMP2, MMP9, and MMP10, and TIMP1 and TIMP2 in CSF and plasma of 52 AD patients (26 without and 26 with microbleeds), 26 matched controls and 24 VaD patients. We found higher plasma MMP2 and CSF MMP10 levels in AD patients compared to VaD patients and controls, and an association of these MMPs with CSF tau and p-tau levels. Secondly, we found decreased concentrations of CSF MMP9, TIMP1 and TIMP2 according to number of microbleeds in AD patients, suggesting their particular involvement in CAA pathology.

In the study in chapter 8 we investigated CSF neurogranin, a post-synaptic protein, as potential novel biomarker for AD. Synaptic degeneration is known to be an early event in AD, but biomarkers for this process were not available yet. This study was a collaboration with the research group of Prof. Blennow and Prof. Zetterberg (Sahlgrenska University, Mölndal, Sweden) who developed a hybrid immunoaffinity mass spectrometry (HI-MS) and enzyme-linked immunosorbent assay (ELISA) method for detection of neurogranin in CSF. We measured CSF levels of this protein in three independent cohorts of patients with AD dementia ($n=100$ in total), mild cognitive impairment patients (MCI) ($n=40$) and controls ($n=80$ in total). First, HI-MS showed that there were several different short C-terminal peptides of neurogranin present in CSF, while the full length protein was not found. Second, we found that these C-terminal fragments of neurogranin in CSF were markedly increased in AD patients compared to controls. Moreover, a high level of CSF neurogranin predicted conversion from MCI to dementia due to AD, and was associated to decline in MMSE over time, as a continuous, more subtle measure of cognitive decline.
2.2 Discussion of the findings

The importance of APOE4 in AD pathology

APOE4 is the most important genetic risk factor for sporadic AD; APOE4 carriers develop AD at a higher annual rate and a younger age than non-carriers. Numerous studies have shown that APOE4 carriers, even subjects without cognitive impairment, have lower levels of CSF Aβ42, and higher Aβ42 deposition on PET-scans. A widely accepted theory supporting these findings is that APOE4 is directly related to increased amyloid deposition. There are three different isoforms of the APOE gene, ε2, ε3 and ε4, leading to three slightly variants of the protein ApoE. ApoE is an important lipid transport protein in the brain. It is able to bind Aβ and is needed for Aβ clearance from the brain. Evidence is accumulating that the ε4 variant of the protein has less binding capacity than ε2 and ε3, and therefore APOE4 carriers are less efficient in clearing Aβ. Carriership of the APOE4 allele is hence thought to promote amyloid accumulation, leading to AD. On the other hand, APOE4 has also been associated with cardiovascular and cerebrovascular disease and worse outcome after traumatic brain injury and subarachnoidal hemorrhage. In addition, several studies reported deleterious effects of cardiovascular diseases on cognition and subsequent cognitive decline over time especially in APOE4 carriers. As such, there is also evidence for a non-specific, and ‘amyloid-independent’ pathway, leading to more systemic cardiovascular disease, increased cerebral vulnerability or impaired repair mechanisms.

In chapter 5 we found that APOE4 increased the risk of cognitive decline along the AD continuum as early as in the stage of subjective complaints. The fact that APOE4 carriers more often had abnormal CSF Aβ42 than non-carriers supports the theory that APOE4 promotes amyloid pathology. However, having only abnormal Aβ42 did not increase the risk of cognitive decline in APOE4 carriers, while it did substantially increase the risk in non-carriers. Only when both Aβ42 and tau were abnormal, APOE4 carriers showed a higher risk of cognitive decline compared to having normal biomarkers. On the other hand, APOE4 carriership, without biomarker abnormalities, may give a slightly higher risk for cognitive decline on its own. Similar results have been found in MCI subjects, in a previous study by our group. These findings therefore suggest that, in addition to promoting amyloid accumulation, APOE4 has an amyloid-independent effect on cognition as well and is an independent risk factor for cognitive decline. An isolated low Aβ42 seems to be insufficient to further increase the risk APOE4 already contains. There has to be neuronal damage – in our study measured by abnormal CSF tau – to further increase risk of cognitive decline in APOE4 carriers.

Subsequently, an important question is why APOE4 increases the risk of cognitive decline. Studies in APOE4 and APOE3 transgenic mice showed that APOE4 enhanced and prolonged brain inflammation in reaction to injection with the pro-inflammatory substance lipopolysaccharide (LPS). In addition, impaired CNS repair mechanisms, or neural
plasticity, have been found in APOE ε4 carriers. Finally, oxidative stress seems to be more important in APOE4 carriers compared to non-carriers. In chapter 6 we found a larger increase over time in the CSF level of F2-isoprostanes, a marker for lipid peroxidation, in APOE4 carriers compared to non-carriers, and only in APOE4 carriers F2-isoprostanes were associated to cognitive decline. This study therefore supports the theory that oxidative stress is indeed one of the amyloid-independent mechanisms contributing to cognitive decline in APOE4 carriers.

In conclusion, APOE4 is an independent risk factor for cognitive decline, even in the earliest AD stage. It exerts its detrimental effects on the brain probably by influencing the amyloid pathway as well as initiating a higher burden of non-specific processes such as oxidative stress. It is plausible that all processes in which APOE4 is implicated can be placed under one denominator, such as the lower binding capacity of ApoE4 (not only for Aβ but also for other proteins), or lower circulating levels of ApoE in APOE4 carriers. Whether this is indeed the case has to be investigated in future studies.

**Microbleeds and blood-brain barrier in AD**

Microbleeds are found much more often on MRI scans of AD patients compared to cognitively healthy subjects, while CAA, of which microbleeds are supposedly a biomarker, is present in the vast majority of AD brains at autopsy. Especially lobar microbleeds seem to be related to CAA, while microbleeds in deep brain locations are more strongly related to vascular risk factors. Microbleeds in AD patients are important from a clinical point of view for several reasons. First, CSF Aβ42 is generally lower in AD patients with microbleeds compared to those without, suggesting a difference in the amyloid pathology in these patients. Second, microbleeds in AD lead to a higher mortality rate, and may be related to worse cognitive performance. It is at present not known why microbleeds have these effects. However, there is circumstantial evidence for blood-brain barrier (BBB) involvement in CAA. Decreased BBB integrity has been found in CAA-affected human brain tissue, as measured by fibrinogen leakage and a decrease in tight junctions. In addition, CAA is associated with spontaneous cerebral inflammation with vasogenic edema, which seems to be caused by a transient increase in BBB permeability. Studies on BBB involvement in AD are inconsistent however, while in VaD patients there is more consistent evidence of increased BBB permeability.

In the study described in chapter 7 we investigated MMPs and TIMPs, proteins involved in BBB breakdown, in plasma and CSF of AD patients with and without microbleeds. We also included VaD patients – most of them having microbleeds as well –, as we were interested in whether the effects of microbleeds on MMPs and TIMPs were more related to CAA or ischemic cerebrovascular damage. Our study showed that MMP2 and MMP10 seem to be mostly related to AD pathology, as the levels of these MMPs were elevated in AD patients but not in VaD patients, and correlated with the levels of CSF markers of neuronal damage tau and/or...
p-tau. On the other hand, TIMP1 and TIMP2 could be related to concurrent CAA pathology in AD patients, as these were decreased in a dose dependent relation with increasing number of microbleeds. These findings suggest that AD patients with microbleeds have less inhibition of MMPs, and therefore more BBB breakdown. This hypothesis could in part explain why the previous studies on BBB permeability in AD are inconsistent; AD patients in these studies probably consisted of a mix of AD patients with and without concurrent CAA, thus with varying degrees of BBB involvement.

In conclusion, it is important to consider microbleeds in AD patients. Presence of microbleeds may have consequences for prognosis and response on (experimental) therapy, due to BBB damage. Whether TIMPs could be more directly linked to BBB damage in AD patients with concurrent CAA, or may be a therapeutic target for these patients is subject for further study.

**AD as a disease of the synapse**

Synapses are the junctions between neurons where communication takes place. The scientist Ramón y Cayal suggested already more than a century ago that dementia could result from damage to the synapses. In the eighties and nineties it was indeed shown that there was a larger decrease in the proportion of synapses compared to that of neurons in brains of AD patients. Moreover, the number of synapses was found to be decreased already in an early stage of the disease and to correlate with dementia severity and neuropsychological test scores. There was until recently no fluid biomarker available for synaptic damage, although several synaptic proteins had been quantified and found to be decreased in AD brains. One of these proteins is neurogranin, which we investigated in the study in chapter 8. Neurogranin is a post-synaptic protein, mainly present in dendritic spines. It is thought to be involved in synapse activity and plasticity, by regulation of the Ca²⁺/Calmodulin pathway, which is in turn important for long-term potentiation.

It had previously been shown that neurogranin was present in CSF. Building on these previous studies, we found that full-length neurogranin, although present in brain, was not present in CSF, while in CSF several different C-terminal peptides were present. In addition, this study was the first to describe that the concentration of neurogranin in CSF was markedly increased in AD patients compared to controls and correlate with levels of CSF tau and p-tau. Most importantly, CSF neurogranin was already increased in MCI patients, and was highly associated with decline in MMSE over time and conversion to dementia due to AD in these patients. This study therefore supports previous findings of the importance of synaptic damage in early stages of AD. CSF neurogranin may have potential as a biomarker for synaptic damage, in addition to the ones we already have for amyloid pathology – Aβ42 – and neuronal damage or neurofibrillary tangle pathology – tau and p-tau.
3. Clinical implications and recommendations for future research

Where do CSF biomarkers fit in?

Based on the results of the studies in the first part of this thesis recommendations could be made on implementation of CSF biomarkers in the diagnostic routine of memory clinics, summarized in the flow chart on page 177. First, we recommend that $\text{A}^\text{B}42$ and tau should be combined in a ratio to make use of their complementary value. It is however important to realize that even this ratio may not always be sufficient, as an abnormal ratio will result from an $\text{A}^\text{B}42$ level of $>1000\text{ pg/mL}$, if tau levels are clearly abnormal. With $\text{A}^\text{B}42$ levels in this evidently normal range, another neurodegenerative disorder is perhaps more likely than AD. The precise cut-offs of the individual markers above which the ratio should not be used anymore are to be determined in future studies.

Second, we could make recommendations based on the identified patients in which CSF biomarkers have added value in the diagnostic process. Most importantly, the use of CSF biomarkers should be considered when diagnostic confidence is low, or when there is an atypical presentation or diagnostic dilemma (i.e. unclear diagnosis), and biomarker results are expected to change patient management. We have also shown that appropriate use criteria for amyloid-PET cannot simply be applied to CSF biomarkers. Either one set of criteria including both biomarker modalities, or separate criteria for CSF biomarkers should be developed.

Third, recommendations can be made on the performance of the LP. Lumbar puncture is a safe procedure, especially when the identified risk factors for post-LP complaints are considered and, if possible, counteracted. A small diameter, atraumatic needle should be used, especially in young patients. Explanation of the procedure with reassurance of the patient could possibly lead to less anxiety and therefore to less post-LP complaints. It could be argued whether a PET-scan is less invasive compared to an LP, as for a PET-scan a radioactive tracer has to be administered intravenously, and patient experience of a PET-scan has not yet been quantified. Moreover, measurement of CSF biomarkers is relatively inexpensive compared to a PET-scan, and recent studies show that results of amyloid imaging and CSF biomarker measurements have a high rate of concordance. Therefore, if a PET-scan could be avoided for the majority of patients by making use of CSF biomarkers instead, a substantial cost reduction could be gained, especially regarding the low incidence of post-LP complications needing medical intervention (i.e. possibly leading to extra healthcare costs). Moreover, CSF analysis also includes tau as a measure of general neurodegeneration, which lacks in amyloid imaging but could give important additional information. One could think of ‘appropriate use criteria’ including both CSF biomarkers and amyloid-PET imaging, in which is stated that CSF biomarkers should be applied first. Only when these biomarkers cannot be obtained (for example due to contraindications for LP, or refusal of the patient) or results give
rise to doubts, amyloid-imaging should be considered as extra evidence for the presence or absence of AD pathology.

**Future directions**

In addition to practical, clinical implications, this thesis also gives rise to new hypotheses and perspectives. We know that AD is not one, simple disease caused by accumulation of one protein. The disease presents differently in different patients, depending on for example presence of the APOE4 allele and presence of microbleeds. Moreover, Aβ42 accumulation may be a starting point or triggering event of the disease, but reactive inflammation, oxidative stress, blood-brain barrier damage, synaptic degeneration and neuronal death are most likely the driving processes in symptomatic stages of AD. When realizing this, it is perhaps not remarkable that none of the trials targeting Aβ plaques in symptomatic stages of AD have shown any effect; clearing Aβ from the brain in this relatively late stage of the disease is probably not effective anymore. Therefore, trials targeting Aβ should be performed in the earliest recognizable or even in an asymptomatic stage of AD. Asymptomatic patients with known autosomal-dominant AD mutations would be in a way an ideal group for this kind of trials, as they will inevitably develop symptomatic AD. A first trial in such patients is currently being performed. In addition, a trial in cognitively normal subjects with evidence for AD pathology on amyloid-PET imaging is being performed.

In addition, we need longitudinal, observational studies with extensive follow up, especially when studying early stages such as MCI or subjective cognitive decline. These studies should preferably be performed with serial CSF and blood sampling. The exact diagnostic and prognostic value of the CSF biomarkers Aβ42, tau and p-tau will only become clear when large cohorts are studied, with follow-up until clinical dementia or death of the patients. Besides, future studies should emphasize on further unraveling all processes that are involved in AD, as it is essential to understand whether certain processes are more important in specific subgroups or stages of the disease. Promising methods for investigating this are proteomics and peptidomics, in which all proteins and peptides in human body fluids such as plasma and CSF are detected and compared between patients and controls. Genome wide association studies (GWAS), searching for susceptibility gene variants, is another possibility. These methods could be applied for detecting differences within AD patients, with the aim to explain heterogeneity in AD, instead of differences between AD patients and control subjects, for which they are currently mostly used. Using these techniques, differences between patients with and without the APOE4 allele, with and without microbleeds, but also groups based on different clinical phenotypes should be explored, as this could help revealing whether there are underlying neuropathological factors we are not yet aware of.

With the help of an extended panel of genetic, biochemical and imaging biomarkers it might eventually be possible to make an individual ‘tailor made’ diagnosis, explaining disease
stage and the most important pathological processes in an individual patient. Although the only window of opportunity for anti-amyloid therapy may be in subjects with preclinical AD, in symptomatic stages of AD a therapy targeting processes beyond amyloid pathology could still benefit the patients. It is plausible that there will never be one single therapy for AD, but that in the future each patient will receive an individualized combination therapy, depending on disease stage and targeted towards specific biomarker abnormalities.
Proposed flow chart for diagnostic assessment of cognitive disorders

**Dementia screening**
- History taking + neurological examination
- Neuropsychological assessment
- Imaging: CT or MRI
- Blood sample: exclude other possible causes

**Clinical diagnosis according to up-to-date criteria**
- Doubts about diagnosis?
- Unclear diagnosis?
- Atypical presentation?
- Patient < 70 years of age?

**AD in differential diagnosis?**
- No
- Yes

**Perform lumbar puncture**
- CSF Aβ42
- CSF Tau

**Ratio tau/Aβ42 > 0.52?**
- No = normal CSF
- Yes = AD profile

**Perform other ancillary investigations**
Based on diagnostic considerations
(E.g. DAT-scan, FDG-PET, referral other specialist)

**Still unclear diagnosis?**
- Aβ42>1000 and tau>500?*

* Exact cut-off levels to be determined.