GENERAL DISCUSSION
SUMMARY AND DISCUSSION

6.1
GENERAL DISCUSSION

GENERAL OBJECTIVE

The overall objective of this thesis was to extend the insight in the clinical applicability of body fluid biomarkers for AD. To this end, we evaluated the biomarkers in relation to several other factors:

> To explore the effects of APOE genotype, in relation to other risk factors, on CSF biomarker levels
> To explore the clinical applicability of CSF biomarkers in differential diagnosis
> To explore the value of CSF biomarkers in relation to AD disease progression
> To develop novel plasma biomarkers for AD

In this chapter, results are summarized and put in perspective

AD and APOE genotype

In chapter 2.1 we examined the associations between APOE genotype and age with CSF biomarker levels. Several articles have been published about the relations between APOE genotype and CSF biomarkers in AD patients and controls, but the results of these studies were often conflicting and were based on small samples. Few studies focused on the combined association of APOE genotype and age on CSF biomarkers, mostly in controls.

In our study, we investigated the associations of APOE genotype and age with CSF biomarker levels of Aβ42, tau and ptau-181 in a large cohort of AD patients and controls. In controls, APOE ε4 carriers had more abnormal levels of Aβ42 (lower), tau (higher) and ptau-181 (higher) than controls without the ε4 allele. For Aβ42 the effects of APOE genotype and age were independent, as both risk factors were associated with lower Aβ42 levels, and these effects were additive. However, there was an interaction between APOE genotype and age for tau and ptau-181: higher tau and ptau-181 CSF levels in APOE ε4 genotype were only seen in older controls. The effects of higher age and APOE ε4 carrierstatus were additive for Aβ42 levels, while higher CSF tau and ptau-181 levels were only seen when both risk factors were present. A possible explanation for this finding is that amyloid pathology develops due to either of these risk factors, while tau/tangle pathology only occurs when both risk factors are present.

In AD patients we found that the relation between APOE ε4 carrierstatus was different for younger and older patients. In younger patients we found the highest CSF tau and ptau-181 levels in the ε4 non-carriers, while in older patients we found the opposite, highest tau and ptau-181 levels were seen in the ε4 carriers. These findings could provide support for the existence of subtypes within AD.

In chapter 2.2 the analysis of a study into the combined effect of hypertension and APOE genotype on CSF biomarker levels was described. Hypertension is associated with an increased risk for AD, however it is not yet clear which mechanisms underlie the association between hypertension and AD. A possible modifying factor in the association of hypertension and AD might be the APOE ε4 genotype. To explore the role of hypertension, in relation to the APOE genotype in the pathogenesis, we examined whether hypertension was associated with CSF biomarker levels of Aβ42,
tau and ptau-181, and whether the APOE genotype modified these associations in a large memory clinic cohort (including patients with subjective complaints, MCI and AD). In this study we found that the associations of the APOE genotype with CSF tau and ptau-181 levels were modified by the presence of hypertension. In APOE ε4 homozygous patients, and to a lesser extent in APOE ε4 heterozygous patients, hypertension was associated with higher levels of CSF tau and ptau-181, whereas in the non-carriers it was not. Hypertension was not associated with Aβ42 levels and APOE genotype did not modify this relation. Our data imply that hypertension is directly related to tau/tangle pathology in APOE ε4 carriers.

In chapter 2.3 we looked into the effect of the APOE genotype in prediction models for progression from MCI to AD using CSF biomarkers. APOE ε4 genotype and abnormal CSF biomarkers have both been reported as predictors of cognitive decline in MCI patients, however their joint effect was not yet clear. In our study we aimed to examine the combined value of CSF Aβ42 and tau levels in relation to APOE ε4 genotype, as predictors for progression to AD in a group of MCI patients. With this study we confirmed previous studies in which both Aβ42 and tau were good predictors. In addition, we found that the APOE ε4 carrier status was a moderate predictor for progression. Furthermore, we found that the predictive value of CSF Aβ42 was higher in APOE ε4 non-carriers, than in ε4 carriers. In ε4 non-carriers progression was associated with lower levels of Aβ42, whereas in the ε4 homozygous carriers progression was independent of Aβ42 levels. By contrast, the predictive value of CSF tau levels was independent of the APOE genotype.

In the study described in chapter 2.4 we focussed on the role of microbleeds. Recent findings indicate that microbleeds are relatively common in the general elderly population, and are even more frequently observed in AD patients. The clinical significance of microbleeds in the pathogenesis of AD remains elusive. Microbleeds are presumably related to amyloid plaques near vessel walls, additionally they have been related to vascular disease. Since APOE ε4 genotype is related to increased amyloid deposits and is a risk factor for vasular disease, it was assumed that microbleeds are more prevalent in ε4 carriers, than in ε4 non-carriers. We investigated in AD patients the associations of microbleeds with clinical, neuropsychological and MRI characteristics, the APOE genotype and levels of cerebrospinal fluid (CSF) biomarkers. We found that having multiple microbleeds was strongly, though non-significant, associated with the APOE ε4 homozygous carrier status. In addition, multiple microbleeds were associated with lower levels of Aβ42. Furthermore, patients with multiple microbleeds had more pronounced cognitive impairment in several cognitive domains. These results suggest that APOE ε4 carrier status increased the risk for the occurrence of many microbleeds, subsequently these microbleeds could play a role in cognitive deterioration. Microbleeds, could thus be important in the risk mechanism of APOE ε4 carrier status for AD.

Discussion of the findings on AD and APOE genotype
The most widely accepted theory with regard to APOE genotype in the pathogenesis of AD lies within the so-called ‘amyloid cascade hypothesis’. According to this hypothesis, it is assumed that the development of AD is initiated by abnormal cleavage of the amyloid precursor protein (APP), resulting in increased availability of
amylloïd. The APOE ε4 genotype is considered a risk factor since it is associated with increased production and decreased clearance of amylloïd, which leads to increased availability of amylloïd beta in the brain.\textsuperscript{22-24} In turn this leads to increased accumulation of amylloïd in plaques. Tangles containing tau are formed in response to amylloïd plaques in a later stage. The combined effect of amylloïd plaques and tau tangle pathology leads to neurodegeneration, i.e. loss of synapses, that lead to the clinical symptoms of AD. The role of the APOE genotype is considered as an up-regulator of this process, as shown in Figure 1.

There are however several other hypotheses regarding the mechanism of APOE ε4 genotype as risk factor for clinical AD. First, the APOE ε4 genotype has been associated with an increased risk for cardiovascular problems, which in turn have been shown to be related to clinical AD.\textsuperscript{25,26} Secondly, inflammation is regarded as a contributor to AD pathology and has been shown to be dependent on APOE ε4 genotype.\textsuperscript{27-29} Thirdly, recent studies showed that APOE ε4 mice models had an impaired synaptic plasticity in the cortex and hippocampus following environmental stimulation, in comparison to APOE ε3 mice, indicating that the APOE ε4 genotype seems less able to adjust its cognition to a more challenging environment.\textsuperscript{30}

In chapter 2, we found support against an exclusive role of APOE ε4 as upregulator of amylloïd beta, while our studies were in line with the proposed alternative hypotheses on the role of APOE ε4 in the pathogenesis of AD. We revealed that the combination of higher age and APOE ε4 carrierstatus in cognitively healthy subjects was associated with higher tau and ptau-181 levels, whereas these factors were independently associated with lower Aβ42 levels. In chapter 2.2 we showed that the combination of hypertension and APOE ε4 carrierstatus was associated with higher levels of tau and ptau-181, whereas this was not seen for Aβ42. From these data it seems that APOE ε4 carrierstatus independently leads to a decrease of Aβ42, while the increase of tau and ptau-181 is also dependent on other risk factors, like older age or hypertension. It could be hypothesized that amylloïd alone is not sufficient for the development of tangle pathology, but that other risk processes are also required in these APOE ε4 carriers, suggesting a more multifactorial etiology. In chapter 2.3 we showed that the predictive value of CSF Aβ42 for progression to AD was modified by APOE genotype. An explanation for the less strict relation between clinical progression and Aβ42 (and thus amylloïd) in APOE ε4 homozygosity could be that other damage processes (vascular, inflammation and decreased plasticity) associated with APOE ε4 genotype also play a role. AD, and especially tangle pathology, could in larger extent be multifactorial in ε4 carrying patients than in patient that lack the ε4 allele. Based on the results of our studies, we propose a refined model for the role of the APOE genotype in the pathogenesis of AD, as is shown in Figure 2. In this model, the APOE genotype directly
upregulates the formation of plaques, however indirectly it also upregulates tau tangle formation, by upregulation of other damage processes, e.g. vascular damage, increased inflammation and impaired repair.

The model, as shown in Figure 2, may have consequences for treatment options for AD. It is conceivable that for APOE ε4 carriers (especially for homozygotes) therapeutic strategies should not solely focus on amyloid beta plaque reduction, but also on managing other processes, like vascular risk factors and abundant inflammation, to counteract tangle formation. As an example, a recent publication describing risk reducing effect of NSAID for the development of AD, found that this effect was present only for those with the APOE ε4 genotype. In addition, clinicians should be more aware of hypertension in APOE ε4 carriers, since ε4 carriers could benefit more from antihypertensive treatment in the prevention of dementia. And, in our opinion, treatment trials for hypertension using dementia or cognitive decline as outcome measure should include stratification for APOE genotype to allow for evaluation of the extent to which treatment effects are modified by the APOE ε4 genotype. Furthermore, the model presented could imply that anti-amyloid therapy in humans is more effective in APOE ε4 non-carriers than in ε4 carriers.

The value of CSF biomarkers in differential diagnosis of AD

In chapter 3.1 we explored the value of CSF biomarkers in the setting of a local hospital memory clinic. In research settings high sensitivity and specificity have been shown for differentiation of AD patients from controls based on these markers. Specialists from local hospital memory clinics are becoming increasingly aware of this, and are more often sending CSF samples for analysis to central specialized laboratories, and results are used in their diagnostic process. However the setting in these clinics differs in many ways from a research setting, and from a tertiary referral setting, and it could be questioned whether results of studies from research settings could be generalized to the setting of a local hospital memory clinic. To clarify the effects of these differences, we evaluated the role of CSF biomarker levels in the diagnostic process of a local hospital memory clinic. As outcome measures we used change in diagnosis and change in confidence in the diagnosis. Patients were clinically diagnosed with no dementia, MCI, AD or another type of dementia (i.e. FTLD, LBD, VaD). We found that the CSF biomarker results matched the primary clinical diagnosis in 69%. In the group with an initial diagnosis of AD this was 77%, which was lower.
than could have been expected from the literature\textsuperscript{32,33} whereas in the other diagnostic groups this percentage was even lower. In one out of ten patients the primary clinical diagnosis was changed, and in addition overall confidence in diagnosis increased after the CSF profile was known. The increase in confidence, after the CSF profiles were revealed, was highest in AD patients.

Our results imply that in addition to clinical evaluation, neuropsychological examination and MRI, CSF biomarker levels can help a local hospital memory clinician by increasing the confidence in the diagnosis, especially for confirmation of the diagnosis of AD.

In chapter 3.2 we investigated the additional value of CSF Aβ40 levels in the differentiation of patients with frontotemporal lobar degeneration (FTLD), patients with AD, and controls. Until now, no satisfactory set of biomarkers has been found to distinguish FTLD from either AD or controls.\textsuperscript{35-40} In a preliminary study we found lower CSF levels of Aβ40 in FTLD compared to both AD patients and controls.\textsuperscript{35} In this study we investigated whether measurement of CSF Aβ40, next to the conventional biomarkers Aβ42, tau and ptau-181, had additional value in the discrimination of the three diagnostic groups. We found that CSF Aβ40 levels could aid in the discrimination of FTLD and controls, and to a lesser extent in the discrimination between AD and controls. For the discrimination between AD and FTLD CSF Aβ40 levels were of no added value, contradicting our earlier preliminary study.\textsuperscript{35}

Discussion of findings on the value of CSF biomarkers in differential diagnosis of AD

A clinical diagnosis of AD is made according to clinical criteria such as the NINCDS-ADRDA criteria for AD.\textsuperscript{41} These clinical criteria are not ideal, studies report sensitivities of 79-98% and specificities of 56-84% for NINCDS-ADRDA criteria compared to post-mortem examination, still considered to be the golden standard.\textsuperscript{42-46} Our studies concerned CSF biomarkers that reflect the pathology of AD. The sensitivity and specificity of these CSF biomarkers are reported as 86-92% respectively 86-89% as compared to post-mortem examination.\textsuperscript{47,48} In addition, the CSF biomarkers can be of help in the differential diagnosis of AD. With the use of the CSF biomarkers 82% of the diagnoses could have been made correctly.\textsuperscript{49} In our studies we used the clinical diagnosis as standard and this may be regarded as a limitation. The diagnosis of AD, and other dementia types, should possibly to a larger extent be based on biomarkers for pathology. This would be very important with the development of therapies that intervene in these pathological processes, such as anti-amyloid immunization therapies. Now, new criteria for the diagnosis of AD are developed where the diagnosis of AD is not only based on clinical findings, but also on ancillary investigations, like CSF biomarker measurements.\textsuperscript{50} With these new criteria, the diagnosis of AD would probably in larger extent reflect the pathology of amyloid plaque formation and tau tangle accumulation.

In the results presented in chapter 3.1 there was a substantial group of patients where the clinical diagnosis did not match the profile of the CSF biomarkers, e.g. the patients had a CSF profile that fitted AD but were clinically diagnosed otherwise. Clinically, these patients apparently did not have AD, whereas possibly pathologically they did have AD. In this study there were many patients with other types of dementia,
like FTLD, DLB and VaD.$^{51,52}$ It is known that there is a considerable part of patients that at post-mortem examination appear to have mixed pathology e.g. Lewy Bodies and amyloid plaques/tau tangles. This could be an explanation for the disappointing figures on discrimination in our study.

For AD we now have several biological markers to gain insight in the pathological processes in vivo (e.g. CSF biomarkers but also PIB-PET). However, for other dementia types less specific markers are available to gain insight in vivo in the pathological processes that underlie the clinical presentation. We feel for this reason that the development of specific biomarkers for other types of dementia could be of benefit in the further discrimination of AD.

In chapter 3.2 we examined whether Aβ40 could be such a marker for FTLD, however the specificity for FTLD appeared limited in this study. More effort should be made to develop biomarkers to increase the discrimination of AD from these types of pathologies and preferably these biomarkers should be disease specific. For example, for FTLD changes in the proportion of 4R and 3R tau isoforms could be an interesting candidate,$^{53}$ and for DLB alfa-synuclein could be promising since this protein is mainly found in Lewy Bodies.$^{54,55}$ In addition, Aβ40 might be a possible biomarker for VaD, since it has been shown that vascular amyloid pathology is associated with altered Aβ40 levels.$^{56}$

Progression of AD and CSF biomarkers

In chapter 4.1 we examined the value of CSF biomarkers as predictors of cognitive decline in AD. CSF biomarkers are associated with progression of MCI to AD.$^{13,15,57}$ Results from studies to further cognitive decline in relation to the CSF biomarkers, however, are conflicting, and often have limited statistical power.$^{4,8,58-64}$ It would be equally important to find a biomarker that reflects the course of AD, in order to monitor disease progression and effects of possible future treatment options. To this aim we examined the value of CSF Aβ42, tau and ptau-181 in prediction models for cognitive decline over time, as measured with the MMSE, in AD. We found high tau in combination with less elevated ptau-181, as represented by a low ptau-181/tau ratio, to be the strongest predictor for cognitive decline over time in AD. Low Aβ42 levels, high tau levels and a high tau/Aβ42 ratio were also associated with more rapid cognitive deterioration. Our findings imply that especially high tau levels, without proportionally increased ptau-181 levels, are an indicator for an aggressive form of AD.

In chapter 4.2 we investigated the value of seven biomarkers (Aβ42, tau, ptau-181, iPF2α-VI, NFL, NFH and Aβ40) in a longitudinal follow-up study in order to identify biomarkers that reflect the course of AD. Several previous studies have evaluated CSF Aβ42, tau, ptau-181 as disease stage markers for AD, to investigate whether these markers could be used for the monitoring of disease progression.$^{65}$ However, until now these AD-specific biomarkers showed little effects in longitudinal settings, as shown in a recent meta-analysis.$^{66}$ We hypothesized that less specific CSF biomarkers like Aβ40, isoprostane, NFH and NFL would possibly perform better in a longitudinal setting. We found that levels of isoprostane, NFL, Aβ40 and tau changed over time, whereas levels of Aβ42 and ptau-181 did not change. Levels of NFL decreased over time, and levels of isoprostane, Aβ40 and tau increased. When comparing the effects over time of the measured biomarkers it appeared that the effect of isoprostane was
largest. In addition increase in isoprostane was associated with progression of MCI to AD, and annual change in isoprostane levels was associated with cognitive decline as measured by MMSE, while for the other biomarkers there was no association with cognitive decline. For this reason isoprostane seemed the most promising marker to monitor disease progression in AD. In this study we confirmed that specific markers for AD like Aβ42 and ptau-181 are not valuable for the longitudinal monitoring of AD. These specific markers seem to be state marker for AD, that only show change before the onset of AD. Non-specific biomarkers, by contrast, show longitudinal effects and could be used to monitor disease progression in AD.

Discussion of the findings on progression of AD and CSF biomarkers

The formation of amyloid plaques has been assumed to be an early process in the pathogenesis of AD. Amyloid has been shown to drive the aggregation of tau tangles. It seems however plausible that cognitive decline is not only associated with the core AD pathologies, of amyloid plaques and neurofibrillary tangles. Non-specific processes like neuronal cell death and synaptic failure, seem far more important for cognitive deterioration. This hypothesis is supported by several types of studies. First, amyloid deposition does not correlate well with dementia severity or disease progression. Second, there are many cognitive intact elderly, with profuse amyloid-beta plaques and neurofibrillary tangles in their brain, while conversely, many demented elderly do not have sufficient plaques or tangles for a diagnosis of AD. In addition, vaccination studies with antibodies against amyloid have not yet shown major cognitive improvement, even though the amyloid plaques decreased.

In both studies described in chapter 4 we found further confirmation for the hypothesis raised in the previous paragraph, that in later stages of AD non-specific pathologic processes are most harmful. In chapter 4.1 we showed that patients with high CSF tau, in combination with relatively less elevated CSF ptau-181, had the most aggressive type of AD. CSF tau has in previous studies been suggested as a reflector of the degree of neuronal cell death, and to be more general marker for neuronal damage, whereas CSF ptau has been assumed to reflect phosphorylation and tangle formation. Thus, in this study we showed that a non-specific marker was most related to cognitive decline. In chapter 4.2 we showed that levels of isoprostane, NFL, Aβ40 and tau were associated with disease progression in AD, whereas levels of Aβ42 and ptau-181 were not. CSF Aβ42 and ptau-181 could be considered as specific markers for AD pathology, whereas the other biomarkers reflect ‘AD-non-specific’ pathological processes. We again confirm that CSF tau is associated with further disease progression of AD, as marker of the degree of neuronal cell death. While, other biomarkers, isoprostane and NFL seemed to perform even better. Neurofilaments (NFL) are markers for neuronal cell loss and isoprostane is an oxidative stress marker, and especially these processes seem to be important in the later stages of progression of AD. This implies that once a patient has the core AD pathologies, of amyloid plaques and tau tangles, to a certain extent, other less specific processes are more significant for disease progression and further cognitive decline.
CHAPTER 6.1

Development of new plasma biomarkers for AD

In chapter 5.1 we evaluated plasma Aβ in the differentiation of AD from controls. Plasma Aβ40 and Aβ42 levels have been found predictors for the development of AD in healthy elderly, but these biomarkers had not yet been evaluated for the diagnosis of AD. In our study we determined Aβ40 and Aβ42 plasma levels in a preliminary cross-sectional setting, to differentiate AD patients from controls. To maximize group differences, only AD patients with a pertinent AD CSF-profile, and controls with a normal CSF-profile, were selected. We found no difference in the values of plasma Aβ40 or Aβ42 or Aβ42/Aβ40 ratio between controls and AD patients. In addition, there was no correlation between plasma and CSF levels of Aβ42. Thus, even in a sample of overt AD patients and controls, we could not observe any discriminating value of plasma Aβ. It seems that the clinical relevance of plasma Aβ40 and Aβ42 is limited in the diagnosis of AD, especially compared to the discriminative power of the CSF biomarkers.

In chapter 5.2 we described the use of mRNA expression measurements by Q-RT-PCR for several signaling markers related to inflammation, hematopoiesis and apoptosis. A recent study reported that an 18-analyte multiplexed plasma panel could differentiate AD from controls, using signaling markers. In our study we have measured mRNA expression for nine of these promising biomarkers: CCL5, CSF1, ICAM1, IGFBP6, IL1A, IL3, IL8, PDGFB and TNF. However, expression levels of mRNA IGFBP6, IL1A, IL3 and PDGFB were too low to analyze. ANOVA for repeated measures revealed that mRNA expression levels of ICAM1, IL8, CCL5, CSF1 and TNF were in general, albeit non-significantly, lower in AD patients than in controls. In addition, there was an interaction for diagnosis and mRNA genes, indicating that the pattern of expression of mRNA genes differed by group. Further analyses of individual mRNA genes showed that expression of mRNA CCL5 was lower in AD patients than in controls, while there were no other significant differences between groups. Levels of both CCL5 and TNF were correlated to CSF levels of tau and ptau-181, which adds to the likelihood that these markers indeed reflect a process that is related to AD pathology. Furthermore, both CCL5 and TNF were correlated to MMSE. These results imply that mRNA expression of CCL5, and possibly also TNF, in plasma could be used to help identify AD patients.

Discussion of the findings on development of new plasma biomarkers for AD

In chapter 5 we focused on the development of novel plasma biomarkers for AD. Up to now there were no plasma biomarkers available that perform in the same range of sensitivity and specificity as CSF Aβ42, tau and ptau-181. In tertiary settings CSF biomarkers Aβ42, tau and ptau-181 are often used, and these markers perform well for differentiation of AD from healthy controls. However, these markers are not available in all clinics and obtaining CSF is considered by many as a cumbersome procedure. Efforts have been made to develop a plasma test for AD to overcome these problems.

In chapter 5.1 we showed that plasma Aβ40 and Aβ42 measurements are not suitable for the diagnosis of AD. In addition, we found that there was no correlation between CSF and plasma levels of Aβ42, which implies that the blood brain barrier
is an obstruction for amyloid to cross to the plasma compartment. It seems that a different kind of markers is needed as plasma marker for AD. For further studies on the discovery of novel plasma biomarkers, it should be taken into consideration, that one should either seek a brain derived biomarker that easily crosses the blood brain barrier, or that one seeks a biomarker that reflects a systemic response to the disease. In chapter 5.2 we examined the mRNA expression for several signaling proteins, that could be related to AD. We found that mRNA expression of CCL5 was lower in AD patients than in controls with reasonable discrimination. mRNA expression was correlated to CSF biomarkers for AD pathology and to the MMSE. This makes it feasible that change in CCL5 expression is a systemic response to AD pathology in the brain. CCL5 mRNA expression could be a promising plasma biomarker for the diagnosis of AD, but replication is necessary. In our experience the measurement of mRNA expression using q-RT-PCR was relatively easy to perform, making it possible to examine large groups of possible systemic markers in a relative short period. Further studies should be performed to evaluate more possible systemic markers for AD, in order to establish a panel of plasma biomarkers for AD. For this cause using the method of Q-RT-PCR for mRNA expression seems promising.

REFERENCES


37. Ian H, Van Swieten JC, Leight S, et al. CSF biomarkers in frontotemporal lobar


RECOMMENDATIONS AND CONCLUSION STATEMENTS

6.2
Recommendations for future research: longitudinal studies and development of novel biomarkers

The data explored in this thesis, generated new hypotheses, and supported hypotheses already raised by other studies. We found support for a more multifactorial role of the APOE ε4 genotype in the early pathogenesis of AD. In addition, we found support for the fact that more non-specific processes determine the rate of progression in further stages of the pathogenesis of AD. These hypotheses are however not yet widely accepted and it is, therefore, important to gain more certainty.

The studies on the role of the APOE ε4 genotype were performed cross-sectionally within our memory clinic cohort. The early pathogenesis of AD is probably a process that starts up to decades before patients present with dementia at a memory clinic. To obtain further insight in the chronology of the processes involved in the early pathogenesis of AD longitudinal studies seem essential. And now, with the establishment of Aβ42, tau and ptau-181 as markers for AD pathology, and for instance PiB-PET, as possibility to make amyloid pathology measurable, and visible, in vivo, it seems to be the right time to start these kind of longitudinal studies. To this end a cohort of elderly at risk, that are at a stage before the onset of MCI or clinical AD, would have to be followed. At different time points information should be gathered about (possible) risk factors, and different types of body fluids (blood and CSF) should be obtained at intervals. With this kind of data it could be clarified how early CSF biomarkers are decreased, or increased, in relation to the onset of clinical AD. In addition, the relation to APOE genotype, vascular risk factors and factors causing neuronal death, could be examined. This kind of longitudinal study is expensive and time consuming, and demanding for subjects included in the cohorts, however for the understanding of the pathogenesis it could be essential.

In our studies we have focussed on the role of the APOE ε4 genotype in the pathogenesis, however there is a large group of patients without the APOE ε4 genotype, that is affected with AD. Risk genes and other risk mechanisms should be explored further in this group of AD patients. For example, the APO J genotype could be a possible risk gene. Its mechanism in the early pathogenesis of AD, and its relation to other risk factors should be unravelled in the same manner as has been done for the APOE ε4 genotype.

In our longitudinal studies we found support for the fact that more non-specific processes determine the rate of progression in AD.
processes determine the rate of progression in further stages of the pathogenesis of AD, as is illustrated in the lower part of Figure 1. Additional studies are warranted to unravel the roles of the found non-specific disease processes (i.e. oxidative stress, axonal loss, neuronal cell loss) in larger extend. For example, our findings should be examined in relation to MRI results and post-mortem findings. In addition, the role of the APOE ε4 genotype in relation to non-specific disease processes should be explored further. It is feasible that the role of the APOE ε4 genotype is also more directly related to the non-specific disease processes as shown in Figure 2.

Furthermore, it is unlikely that we have examined the whole scope of possible non-specific damage processes. Inflammation probably has an important role in the later stages of the pathogenesis of AD, and for this reason biomarkers for inflammation should be explored further in longitudinal studies as soon as possible. Furthermore, biomarkers for other pathologies that could overlap with AD, should be developed in order to provide further insight in the overlap with pathologies of other dementia types like FTLD, DLB and VaD.

The results of this kind of studies may provide suggestions for treatment options for later stages of AD pathology. From our current findings, it seems unlikely that at the stage of clinical AD the effects of therapies that intervene in amyloid accumulation, like vaccination with amyloid antibodies, would yield much benefit. However, therapies that target the more non-specific pathogenic processes could possibly be of benefit in patients that already are at the stage of clinical AD.4
GENERAL DISCUSSION

General conclusion statements:

» The APOE ε4 genotype has a multifactorial role in the pathogenesis of AD
» Non-specific pathological processes seem most harmful in advanced stages of AD
» Biomarkers for inflammation should be evaluated for the monitoring of disease progression of AD
» Efforts on the development of plasma markers should focus on markers that reflect systemic response to AD pathology
» Disease specific markers for other types of dementia, e.g. FTLD, DLB and VaD, should be developed to further distinguish the different pathologies in vivo

REFERENCES