SUMMARY

Introduction
Biomarkers of AD pathology in CSF and on MRI are now available and can be used to diagnose subjects with ‘MCI due to AD’. However, as the criteria for MCI due to AD have not yet been validated, prognosis of subjects fulfilling these criteria is largely unknown. Aim of this thesis was to assess the use of AD biomarkers for the diagnosis and prognosis of subjects with MCI and for the selection of MCI subjects for clinical trials. The thesis has two main topics: I. Biomarkers as predictors for AD-type dementia in subjects with MCI, II. Biomarkers as predictors of cognitive decline in subjects with MCI due to AD.

Main findings

Biomarkers as predictors for AD-type dementia in subjects with MCI

In chapter 2 we assessed biomarkers in blood, CSF and MRI as predictor for progression from MCI to AD-type dementia and for the selection of MCI subjects for clinical trials.

In chapter 2.1 we performed a meta-analyses of biomarkers for progression from MCI to AD-type dementia in blood and CSF. In addition, we used these results to explore the use of CSF biomarkers for the selection of subjects for a fictive trial with a drug that aims to slow down progression from MCI to AD-type dementia. In blood only Aβ1-40, Aβ1-42 and homocystein have been tested in multiple studies, but none of them could predict progression from MCI to AD-type dementia. In CSF Aβ1-42, t-tau and p-tau all predicted progression from MCI to AD-type dementia. The combination or ratio of CSF Aβ1-42 and t-tau was the best predictor for progression to AD-type dementia, with a sensitivity of 0.87 and a specificity of 0.70. We found that the use of CSF Aβ1-42 and tau for the inclusion of MCI subjects in a clinical trial could substantially reduce trial costs.

In chapter 2.2 we assessed the combination of CSF and MRI biomarkers as predictors for progression from MCI to AD-type dementia and for the selection of subjects for clinical trials. We found that the ratio of Aβ1-42/t-tau in CSF and hippocampal atrophy on MRI independently predicted progression from MCI to AD-type dementia, but the overall predictive accuracy of CSF Aβ1-42/t-tau for AD-type dementia after two years was around twice as high as that of hippocampal atrophy. After analysis of CSF, additional MRI analyses had prognostic value when the Aβ1-42/t-tau ratio was normal, but not when the Aβ1-42/t-tau ratio was abnormal. Analysis of CSF Aβ1-42/t-tau ratio always improved predictive accuracy for AD-type dementia and cognitive decline, regardless of MRI results. The use of only CSF biomarkers for the selection of MCI subjects for a clinical trial had the best trade-off between sample size and number of subjects required for screening.

In chapter 3 we compared the predictive accuracy of biomarkers in CSF and MRI for different MCI subgroups.

In chapter 3.1 we found that the overall predictive accuracy of CSF Aβ1-42 and to a lesser extent CSF t-tau for AD-type dementia was lower in older than in younger MCI subjects. The lower overall predictive accuracy in older subjects was mainly caused by a higher prevalence of abnormal biomarkers in older subjects who did not progress to AD-type dementia (MCI-no...
AD) for both CSF Aβ1-42 and t-tau. In addition, older who progressed to AD-type dementia had less abnormal levels of CSF Aβ1-42 than younger subjects, especially when they were APOE-ε4 negative. The use of age-optimized cut-points did not improve overall predictive accuracy.

In chapter 3.2 we compared the predictive accuracy of CSF and MRI biomarkers in subjects with amnestic and non-amnestic MCI. Overall predictive accuracy for progression to AD-type dementia of both CSF and MRI measures was similar for subjects with amnestic MCI and subjects with non-amnestic MCI, but sensitivity was lower and specificity higher in subjects with non-amnestic MCI. Calculating optimized cut-points for both subgroups separately resulted in more lenient cut-points for subjects with non-amnestic MCI. Although using these cut-points did not change overall predictive accuracy, it increased sensitivity at the cost of a lower specificity in subjects with non-amnestic MCI, resulting in a sensitivity and specificity similar to those in subjects with amnestic MCI.

Biomarkers as predictors of cognitive decline in subjects with MCI due to AD

In chapter 4.1 we selected subjects with MCI who all progressed to AD-type dementia and compared CSF and MRI biomarkers as predictors for cognitive decline. We found that high levels of CSF t-tau and p-tau, medial temporal lobe atrophy and low MMSE score predicted more rapid progression to AD-type dementia, while CSF level of Aβ1-42 was not associated with time to dementia.

In chapter 4.2 we selected subjects with MCI and abnormal CSF Aβ1-42 and assessed whether CSF t-tau and hippocampal atrophy could predict progression to AD-type dementia and rapid cognitive decline. During a mean follow up of 2.3 years, 57% of the subjects progressed to AD-type dementia. For subjects with only abnormal CSF Aβ1-42 but no abnormal injury markers (i.e. normal CSF tau and hippocampal volume) disease course was relatively benign, while almost all of the subjects who had also abnormal CSF t-tau and hippocampal atrophy progressed to AD-type dementia.

In chapter 5.2 we will discuss our main findings and answer the research questions described in the introduction. In addition we will address methodological issues of the studies described. We will conclude with recommendations for future research.

GENERAL DISCUSSION

I. Biomarkers as predictors for AD-type dementia in subjects with MCI

Biomarkers in blood

In chapter 2.1 we performed a meta-analysis of previous studies assessing blood biomarkers as predictors for AD-type dementia in MCI. As beta amyloid (Aβ) is the key pathological feature in AD, several studies have assessed Aβ in blood. However, our meta-analysis showed that neither Aβ1-40, Aβ1-42 nor the ratio of these two could predict AD-type dementia. Apparently, brain metabolism of beta amyloid is not directly reflected in blood, consistent with the finding that blood levels of Aβ1-40 and Aβ1-42 do not correlate with the presence of amyloid plaques in the brain. A possible explanation for this is that the blood-brain barrier hinders diffusion of
proteins from brain to blood. In addition, blood concentrations are derived from whole body metabolism and metabolism in the brain might only account for a part of total variation. Apart from Aβ, homocystein is the only single marker that has been assessed as predictor of progression from MCI to AD-type dementia in multiple studies. Our meta-analysis showed that plasma concentrations were not associated with progression from MCI to AD-type dementia. An interesting approach for blood biomarkers are the proteomics or metabolomics studies, using a combination of proteins to construct a biomarker profile. One study found a combination of proteins that predicted progression from MCI to AD-type dementia with high accuracy, but in a subsequent study to test reproducibility and another recent study results were disappointing. Although a reliable biomarker for AD pathology in blood is highly desired, it seems unlikely that it becomes available at short notice.

**Biomarkers in CSF**

Levels of Aβ1-42, t-tau and p-tau in CSF have been shown to highly correlate with amyloid plaques and neurofibrillary tangles in the brain and predict progression to AD-type dementia in subjects with MCI. In our meta-analysis (chapter 2.1) we showed that CSF Aβ1-42, t-tau and p-tau in CSF all predicted progression from MCI to AD-type dementia (odds ratio’s (OR) between 7.5 and 8.1). The combination or ratio of CSF Aβ1-42 and t-tau was the best predictor for progression to AD-type dementia, with an OR of 18.1 (95% confidence interval (CI) 9.6-32.4), a sensitivity of 0.87 and a specificity of 0.70. Although an abnormal ratio of CSF Aβ1-42 and t-tau thus turned out to have a the best predictive accuracy for progression to AD-type dementia, from a pathophysiological perspective using the ratio might not be ideal as CSF Aβ1-42 and t-tau reflect other pathological processes. For example, an abnormal ratio caused by a high level of CSF tau and normal CSF Aβ1-42 is likely to reflect different pathology than a similar ratio caused by a normal or slightly elevated CSF tau and low CSF Aβ1-42.

A number of other biomarkers, reflecting dysregulation of amyloid metabolism or subsequent processes in AD pathology as neurodegeneration, inflammation, oxidative stress or altered lipid metabolism can be measured in CSF. Their possible use for early diagnosis or prognosis remains to be determined, since only few of them have been tested as predictor from MCI to AD-type dementia in more than one study.

**Hippocampal atrophy on MRI**

In chapter 2.2 we found that hippocampal atrophy predicted progression to AD-type dementia within two years (OR 3.7, 95% CI 1.8-7). Hippocampal atrophy had a low sensitivity for progression to AD-type dementia (0.56) but a rather high specificity (0.74), in line with the suggestion that hippocampal atrophy is a relatively late event in the pathophysiological cascade (figure 1).

**Combination of CSF and MRI biomarkers**

In chapter 2.2 we found that the ratio of Aβ1-42/t-tau in CSF and hippocampal atrophy on MRI independently predicted progression from MCI to AD-type dementia, but the overall predictive accuracy of CSF Aβ1-42/t-tau for AD-type dementia after two years was around twice as high as that of hippocampal atrophy. In clinical practice MRI is often routinely used
to exclude structural lesions as a cause of the cognitive complaints. As we found that analysis of CSF Aβ1-42/t-tau ratio improved predictive accuracy for AD-type dementia and cognitive decline regardless of MRI results, even in those settings CSF analyses can be useful.

**Effect of age and MCI subtype on predictive accuracy of CSF and MRI biomarkers**

**Age**

The overall predictive accuracy of CSF Aβ1-42 and to a lesser extent CSF t-tau for progression to AD-type dementia was lower in older than in younger MCI subjects, but their positive predictive value was not affected by age (chapter 3.1). This means that for MCI subjects with abnormal CSF biomarkers progression rate to AD-type dementia is similar, regardless of age. However, as the prevalence of AD is higher in older individuals, in clinical practice CSF biomarkers may have lower added diagnostic value for older than for younger subjects. The lower overall predictive accuracy in older subjects was mainly caused by a higher prevalence of abnormal biomarkers in older subjects who did not progress to AD-type dementia (MCI-no AD) for both CSF Aβ1-42 and t-tau. As a recent study found a slower cognitive decline in older MCI subjects it could be that those subjects would have eventually progressed to AD-type dementia after longer clinical follow up. In addition, older subjects who progressed to AD-type dementia had less abnormal levels of CSF Aβ1-42 than younger subjects, especially when they were APOE-ε4 negative. The use of age-optimized cut-points did not improve overall predictive accuracy.

**MCI subtype**

In chapter 3.2 we assessed the predictive accuracy of CSF Aβ1-42 and t-tau and hippocampal volume in relation to clinical presentation. Overall predictive accuracy for progression to AD-type dementia of both CSF and MRI measures was similar for subjects with amnestic MCI and subjects non-amnestic MCI, but sensitivity was lower and specificity higher in subjects with non-amnestic MCI. At baseline subjects with non-amnestic MCI had a larger hippocampal volume than subjects with amnestic MCI, consistent with the role of hippocampus in memory performance. Calculating optimized...
cut-points for both subgroups separately resulted in more lenient cut-points for subjects with non-amnestic MCI. Although using these cut-points did not change overall predictive accuracy, it increased sensitivity at the cost of a lower specificity in subjects with non-amnestic MCI, resulting in a sensitivity and specificity similar to those in subjects with amnestic MCI.

A remarkable finding was that both subjects with non-amnestic MCI and older APOE-ε4 negative subjects progressed to AD-type dementia at relatively high levels of CSF Aß1-42. We hypothesize that in those subjects other factors are involved in the pathophysiological cascade, causing cognitive decline already at a lower amyloid load. This finding supports the existence of different AD phenotypes, with a distinct clinical and biomarker profile.

The use of biomarkers for the selection of MCI subjects for clinical trials

In chapter 2.1 and 2.2 we explored the use of CSF and MRI biomarkers for the selection of subjects for a hypothetical trial with a drug that aims to slow down progression from MCI to AD-type dementia. We found that including only subjects with abnormal CSF Aß1-42 and tau would substantially reduce trial costs. Because subjects with abnormal CSF Aß1-42 and tau show more rapid cognitive decline than unselected MCI subjects, less subjects are needed in the trial to establish the same treatment effect. Although this strategy increases the costs for screening, it reduces the overall trial costs. The optimal selection method in research practice will depend on the specific trial characteristics, as for example for a trial with very expensive medication, using a combination of abnormal CSF and MRI could be attractive because this increases the number of subjects to screen but results in a smaller sample size. A possible disadvantage of using CSF for trial inclusion is that it might induce some selection bias, as subjects with contra-indications for lumbar puncture cannot be included. As this excludes for example subjects with vascular problems using anticoagulants from trial participation, generalizability of trial results might be hampered.

II. Biomarkers as predictors of cognitive decline in subjects with MCI due to AD

Using biomarkers, AD can now be diagnosed in subjects with MCI.20,21 As clinical progression in subjects who fulfill these criteria is largely unknown, it is important to define prognostic markers. In chapter 4 we took two different approaches to assess prognostic markers in subjects with MCI due to AD. In chapter 4.1 we selected MCI subjects who all progressed to AD-type dementia during follow up. In those subjects high levels of CSF t-tau and p-tau, medial temporal lobe atrophy and low MMSE score predicted more rapid progression to AD-type dementia, while the CSF level of Aß1-42 was not associated with time to dementia. These findings were interesting from a pathophysiological perspective, but our selection of subjects based on outcome limited clinical applicability. Therefore in chapter 4.2 we took a different approach. For this study we selected subjects with MCI and evidence of amyloid pathology, defined by an abnormal level of CSF Aß1-42. Subsequently all included subjects fulfilled the criteria for ‘MCI due to AD’ and ‘prodromal AD’.20,21 During our study, with a mean follow up of 2.3 years, 57% of these subjects progressed to AD-type dementia. For subjects with only abnormal CSF Aß1-42 but no abnormal injury markers (i.e. normal CSF tau and hippocampal volume) disease course was relatively benign, while almost all of the subjects who had also abnormal CSF t-tau and hippocampal atrophy progressed to AD-type dementia. This is in line with the order of pathological events
as suggested amyloid cascade hypothesis (figure 1), \(^{17,18}\) as subjects with abnormal injury markers are already further downstream the cascade and therefore more likely to reach threshold for dementia at short notice. However, in chapter 4.2 we found that subjects with abnormal CSF tau levels had similar cognitive functioning at baseline, but showed more rapid cognitive decline compared to subjects with normal CSF t-tau. This indicates that for some subjects the pathophysiological cascade comes along more quickly than for others.

**Methodological issues**

**Clinical aspects**

For the analyses in this thesis we included subjects from the memory clinic based Amsterdam Dementia Cohort and the DESCRIPA study. This multicenter approach enables generalization to other memory clinic populations, although applicability to other settings, such as the general population is limited. Evidently, for the CSF analyses we could only include subjects who underwent a lumbar puncture. This may have led to some selection bias, as we found in chapter 2.2 that subjects who underwent lumbar puncture tended to be younger, more frequently male and had lower MMSE score at baseline.

Diagnoses of MCI and AD-type dementia were made in multidisciplinary teams, according to the current clinical criteria. The teams involved in clinical diagnosis were not aware of CSF results and the teams performing CSF analyses were blinded for clinical information. In most cases the team involved in the clinical diagnosis was aware of MRI results, which may have led to some overestimation of MRI results. We had no pathological confirmation of diagnoses, which may have led to diagnostic misclassification in some cases. In addition, as the follow up in our studies was limited (mean follow up around 2.5 years) it could be that some subjects who were diagnosed with MCI during our follow up later progressed to AD-type dementia.

**CSF**

CSF measures were performed in Amsterdam for the subjects from the Amsterdam Dementia Cohort and in Gothenborg for subjects from the DESCRIPA study. Since ELISA measurements have been shown to vary between centers, \(^{22}\) we corrected for interlaboratory ELISA differences by means of 33 samples that were analyzed in both laboratories and adjusted the values of the Amsterdam Dementia Cohort to those of DESCRIPA. Currently there are no uniform international cut-points to define abnormal CSF Aβ1-42, t-tau and p-tau, which have led to some variation in cut-points used throughout this thesis. However, as we showed in some of our studies that using different cut-points had no major effect on predictive accuracy, the influence of the use of different cut-points on our overall conclusions is most likely limited. For some analyses we calculated cut-points and chose the optimal cut-point based on the highest Youden index (sensitivity+specificity-1). As the Youden index is composed of both sensitivity and specificity, the highest Youden index can for one marker be based on a high sensitivity and for another marker on a high specificity. In practice choosing a cut-point with either a high sensitivity (to avoid missing AD cases) or a high specificity (to prevent diagnosing subjects that remain clinically stable with AD) could be preferable, depending on the specific setting. The predictive accuracy we found for the CSF markers in our meta-analysis were higher than
those in our own studies. This may be due to several reasons. Some of the studies included in the meta-analyses had longer clinical follow up than our own studies. In addition, some studies used data driven cut-points for the CSF markers. Furthermore some studies defined abnormal CSF as an abnormal ratio of CSF Aβ1-42/t-tau, while others used a combination of two abnormal biomarkers to define abnormal CSF. Finally there may have been some publication bias, resulting in an overestimation of the predictive accuracy in our meta-analysis.

**MRI**

In this thesis we used two MRI measures for hippocampal atrophy. In chapter 4.1 medial temporal lobe atrophy (MTA) was rated visually, according to the method of Scheltens et al. For chapter 2.2, 3.2 and 4.2 we measured hippocampal volume (HCV) using learning embeddings for atlas propagation (LEAP). Both 1.0 and 1.5 Tesla MRI scans were used, depending on the participating center. As field strength did not affect the LEAP score (chapter 4.2) we used data from both field strengths without correction. The effect of field strength on MTA score has not been described, but as changes between 1.0 and 1.5 Tesla are minimal, it is unlikely that field strength would have a major effect on the visual rating scale. Both MTA and LEAP scores have been shown to predict AD-type dementia, although a recent study showed that hippocampal volume measured with LEAP has a higher predictive accuracy for AD-type dementia than MTA scores. It is therefore possible that in chapter 3.1 were we used the visual MTA score we underestimated MRI results.

**Concluding remarks**

**I. Biomarkers as predictors for AD-type dementia in subjects with MCI**

Although a reliable biomarker for AD pathology in blood is highly desired, it seems unlikely that it becomes available at short notice. At this moment, the combination of CSF Aβ1-42 and t-tau is the strongest predictor for progression from MCI to AD-type dementia (figure 2). Using these CSF biomarkers for the selection of MCI subjects for clinical trials substantially reduces sample size and costs. Hippocampal atrophy also predicts progression to AD-type dementia, but sensitivity for progression within the subsequent years is lower than that of the CSF markers. In older subjects overall predictive accuracy of CSF Aβ1-42 and t-tau is lower due to a higher prevalence of AD biomarkers in clinically stable subjects, but even in elderly subjects AD pathology should not be considered benign, as we found that the positive predictive value of the biomarkers was not related to age. Older APOE-ε4 negative subjects and subjects with non-amnestic MCI can progress to AD-type dementia with relatively high levels of CSF Aβ1-42. Using different cut-points in for the different MCI subgroups, did not improve overall predictive accuracy, but did cause changes in sensitivity and specificity. These findings suggest that there may be differences in the underlying pathophysiological cascade for different MCI subgroups.

**II. Biomarkers as predictors of cognitive decline in subjects with MCI due to AD**

Of the subjects fulfilling the criteria for MCI due to AD less than 60% had progressed to AD-type dementia after two years (chapter 4.2). We found that for subjects with MCI and amyloid pathology but without biomarker evidence of neuronal injury disease course was relatively benign, while subjects who had also abnormal injury markers showed rapid cognitive decline.
This finding is in line with the respective place of the biomarkers in the pathophysiological cascade of AD (figure 1). We suggest that the diagnostic work-up of subjects with MCI may have a two-step approach. First, amyloid markers, as CSF Aβ1-42 can be used to define whether AD pathology is present. Second, injury markers as CSF t-tau and hippocampal atrophy can be used to determine prognosis (figure 1 & 2).

**Recommendations for future research**

Despite all efforts, a cure for AD is still lacking. In the search for an effective therapy better understanding of the pathophysiological mechanism and intervention in an early stage of the disease seem crucial. The research criteria for MCI due to AD need to be validated.

**Towards a more detailed pathophysiological cascade**

An important reason for failure of therapy so far is the lack of detailed understanding of the pathophysiological cascade in AD. We found differences in biomarker profiles in MCI subgroups based on age and clinical presentation, supporting the existence of different phenotypes. Identifying those is crucial for defining prognostic markers for individual subjects and eventually for establishing an effective therapy. Special attention should be paid to subjects with abnormal AD biomarkers who do not progress to dementia at short notice. Identifying the factors protecting those subjects from cognitive decline despite AD pathology, may improve understanding of the disease. As most likely factors beyond the scope of this thesis, e.g. inflammation, vascular factors and oxidative stress play a role in the pathophysiology of AD, the search for new biomarkers should continue.

**Trials in the predementia stage of AD**

As one of the reasons for the failure of previous trials may be that they were performed in the dementia stage of AD, the new research criteria that allow an AD diagnosis in subjects with MCI
seem promising. At this moment trials are ongoing trying to modify amyloid pathology in subjects with MCI and evidence of amyloid pathology. For future studies it may be beneficial to also use CSF t-tau for the inclusion of subjects, as we showed that CSF Aβ1-42 can be used as a marker for early AD diagnosis, but CSF t-tau is a better prognostic marker for cognitive decline at short notice. In addition, stratification of subjects based on clinical and biomarker characteristics, as is already done in some AD trials for APOE genotype, may improve treatment strategies and drug development.

**Validation of the research criteria for MCI due to AD**
As we found that many subjects fulfilling the research criteria for MCI due to AD had not progressed to AD-type dementia after two years, validation of the criteria in studies with longer clinical follow up is needed. Since we found differences in sensitivity and specificity of the CSF and MRI biomarkers based on subjects’ age and clinical presentation, the use of different cut-points for specific MCI subgroups needs to be explored further.

**REFERENCES**


