SUMMARY

In this thesis the diagnosis of Alzheimer Disease in a memory clinic setting is described, focusing on the use of biochemical markers (Chapter 1 and 2). The most important biomarkers for the diagnosis are Aβ42, Tau, and Ptau in CSF. These biomarkers are derived from the characteristic hallmarks of AD, the senile plaques and the neurofibrillary tangles in brain. However, other pathological processes, associated with AD, are also studied to obtain more insight in the etiology of the disease.

The most important finding was that the post-mortem hallmarks of AD provide ante-mortem biomarkers for the diagnosis. The use of biomarkers Aβ42, Tau and Ptau was studied using commercial ELISAs in CSF from AD patients and patients with subjective memory complaints. Inter-assay CV's were about 10% for the three parameters. The cut-off values were 550 ng/L for Aβ42, 375 ng/L for Tau, and 52 ng/L for Ptau at a chosen sensitivity of 85%. Corresponding specificities were, respectively 83%, 78%, and 68%. Logistic regression to investigate the simultaneous impact of the three CSF biomarkers on the diagnosis yielded a sensitivity of 94% and specificity of 83%, however, in this setting Ptau did not have additional value. (Chapter 11).

At the start of these investigations, preanalytical influences on the levels of the CSF biomarkers to be studied were largely unknown. We, therefore, investigated the stability of the biomarker levels, also from the perspective that in the beginning accrual of samples was low and specimens had to be stored prior to analysis. Stored for a long period at -80°C the concentrations of Aβ42 and Tau in CSF are remarkably stable. CSF Aβ42 decreased by 20% during the first 2 days at 4, 18 and 37°C compared with -80°C. CSF Tau decreased after storage for 12 days at 37°C. After three freeze/thaw cycles, CSF Aβ42 decreased 20%, and CSF Tau was stable during six freeze/thaw cycles. However, freezing/thawing of fresh CSF samples did not have any influence. In addition, centrifugation did not influence the biomarker concentrations (Chapter 3).

The Aβ42 concentrations were measured by two different assays in the same CSF samples (‘split samples’) on two locations. The first assay is widely used in Europe while the second assay is used mainly in the United States of America. The concentrations, measured by both methods did not differ statistically significant from each other. In AD patients vs. controls, the sensitivity and specificity were 90%, for both assays. Comparing the AD and FTLD patient groups, we obtained specificity of 71% at a sensitivity of 85%, with a trend of better discrimination for the USA method (p =0.045) (Chapter 4).

In Chapter 5 we studied two new potential markers SAP and C1q, which showed comparable CSF levels in AD patients and controls. For CSF-SAP and CSF-C1q conflicting results have been reported, perhaps due to the use of different control groups. More likely, however, there are differences in sensitivity and specificity of the antibodies used in the assay methods. In our study, well defined and documented AD patients and healthy controls were compared, yielding a large ‘black and white’ contrast, probably more adequate to investigate the potential value of a new biological marker. Our
results render these new markers probably useless for AD diagnosis. In **Chapter 6**, CSF concentrations of the NOS inhibitor ADMA and its hydrolysis product dimethylamine in AD patients were studies and showed no difference when compared to age-matched control subjects. In addition, concentrations of arginine, the substrate of NOS, and SDMA also did not differ between AD patients and controls. These results suggest that in AD there are no alterations of NOS activity on the concentration of either substrate or the endogenous inhibitor ADMA.

In **Chapter 7**, no difference in total PC concentrations was found between AD patients and controls. The lyso-PC/PC ratio was significantly decreased in CSF of AD patients, suggesting alterations in the metabolism of choline-containing phospholipids in the brain in AD, but the difference between both groups was small and considerable overlap was present, also rendering it less useful as biomarker for AD.

The level of the metabolites of the transmethylation cycle in CSF of AD patients was similar to that of controls (**Chapter 8**). These findings argue against a possible change in methylation of the promoter and expression of PS1. Deposits of Aβ in plaques in AD brain may result from posttranscriptional or posttranslational changes in PS1 activity rather than from over expression of the PS1 gene by undermethylating of its promoter.

In **Chapter 9**, a strong relationship between white matter changes on MRI (WMH) and low plasma vitamin B6 levels in patients with AD was identified. Homocysteine, which is partially metabolized through the transsulfuration pathway, where homocysteine condenses with serine to cystathionine in a vitamin B6-dependent reaction, may mediate the observed effect of B6 on WMH. Consequently, low vitamin B6 levels may cause high homocysteine levels, thus promoting the proliferation of smooth muscle cells and initiating or accelerating the progression of atherosclerosis, which is related to WMH.

The previously demonstrated inverse relationship between plasma vitamin B6 concentrations and grade of WMH in the brain of AD patients was confirmed in **Chapter 10**. Only vitamin B6 level was linked to WMH, whereas homocysteine, vitamin B12, and folate were not. Antioxidant properties, especially the strong quenching effect of the pyridoxine moiety within vitamin B6 on singlet molecular oxygen, may explain the inverse relationship between plasma vitamin B6 and WMH.