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Biochemical markers in dementia

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Chapter 1

General introduction and aims of the thesis

1.1

General introduction

Alzheimer's disease in the clinic

A diagnosis of probable Alzheimer's disease (AD) is made by clinical criteria established by McKhann and colleagues¹. Patients should have progressive worsening of memory and dysfunction in at least one other area of cognition (orientation to place and time, memory, language, praxis, attention, visual perception or executive function), in the absence of delirium¹. Other systemic disorders and brain diseases that could account for the progressive deficits in memory and cognition should be excluded; if not the designation is possible AD.

Research in AD: early/differential diagnosis and follow up

In the near future the number of AD patients is expected to increase (see Figure 1)^{2, 3}, however, the diagnostic accuracy of the aforementioned clinical criteria is relatively low (sensitivity 80% and specificity of 70%⁴). With this in mind, biological markers are needed now more than ever. Not only as an aid in early diagnosis or for differential diagnosis, but also to monitor progression of this disease.

In the past two decades, a vast amount of research has been performed to develop biomarkers that can detect patients with cognitive dysfunction who are not demented yet. These patients, who are at increased risk for developing AD, are diagnosed by using the term mild cognitive impaired (MCI)⁵. MCI is the most commonly used term for individuals who have subjective memory complaints or other cognitive symptoms, objective memory or cognitive impairment and whose activities of daily living are generally normal and thus are not considered to be demented. Progression to dementia occurs at a higher rate (50% in 4 years) from MCI patients than from an unimpaired state⁶. For this reason, it is important to identify these subjects, which makes it possible to detect AD in early stages.

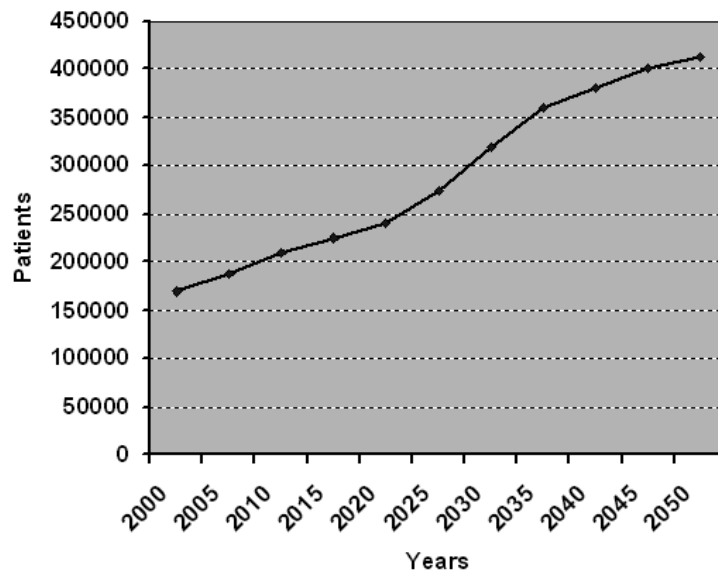
The research field not only focuses on early diagnosis, but also on the development of biomarkers that can be used in differential diagnosis (see Figure 2)^{7, 8}. Many MCI patients have incipient AD, however, others probably have a benign form of memory impairment or have other neurodegenerative diseases that eventually cause dementia [e.g. vascular dementia (VD)⁹, frontotemporal lobar degeneration (FTLD)¹⁰, Lewy body disease (LBD)¹¹]. For treatment and estimation of prognosis, it is important to differentiate between the different causes of dementia.

Besides a the great need for biomarkers for early and differential diagnosis, the last years several biomarkers have been investigated to use for follow-up of patients¹²⁻²⁵. Since the remarkable results achieved by immunotherapy in mice²⁶, biomarkers that can monitor disease progression or remission are considered to be potential useful.

Pathogenesis of AD

Neuropathologically, AD is characterized by senile plaques and neurofibrillary tangles^{27, 28}. The main constituent of the plaques is the amyloid-beta protein (A β)^{29, 30} and the neurofibrillary tangles mainly consist of abnormal hyper-phosphorylated Tau (P-Tau) and Tau proteins (see Figure 3)³¹. It is believed that the accumulation of A β [from oligomers (small A β -aggregates), to fibrils (large A β -aggregates)] in the limbic system and association cortices, occurs prior to formation of plaques and tangles and initiates a complex cascade of biochemical and cellular changes that culminates in memory and cognitive impairment³²⁻⁴⁶. This will eventually lead to cell death (See Figure 3 and 4).

Figure 1



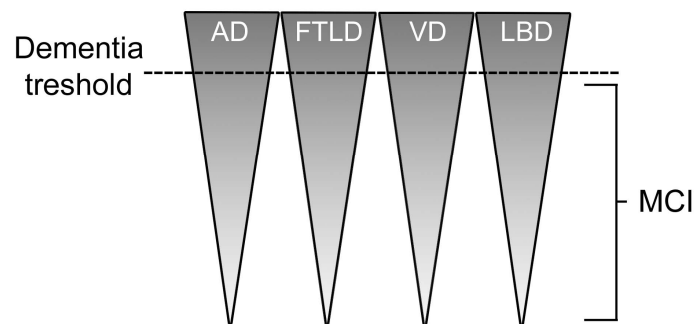
Actual and estimated number of new AD cases in The Netherlands through the year 2050³.

Composition of A β plaques and the immunohistochemical detection

As mentioned, diagnosis of AD is most often made clinically. A definite diagnosis, however, can only be made post-mortem. One of the most striking neuropathological features of AD is partly based on the detection of A β deposits (as diffuse or cored plaques, or in the blood vessels in the cortical areas of the brain)^{27, 28}. For this reason, the assessment of A β in brain tissue has become an important facet of diagnostic practice and studies investigating the role of A β in AD pathogenesis. The amyloid deposits vary in protein composition in terms of the types of A β peptides^{47, 48}. A β peptides found in plaques are 39 to 43 amino acids in length and occur in two major forms: A β 42 and A β 40^{49, 50}. The N-terminus of these A β peptides is known to be heterogeneous^{50, 51}. Further, next to A β , other proteins accumulate within senile plaques (associated factors), including several inflammation factors, apolipoprotein E (apoE), SAP and α 1-antichymotripsin (ACT)^{50, 52, 53}. The function of these associated factors is not known yet.

Detailed instructions of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD)⁵⁴ and the Braak and Braak staging of AD-related^{27, 28, 55} changes, are recommended by the National Institute of Aging and Reagan (NIA-Reagan) Working Group^{56, 57} to estimate AD pathology (See Table 1). These recommendations include also specific immunohistochemical (IHC) stainings which make the diagnosis of AD more accurate and reliable⁵⁷. However, no consensus exists on which antibodies have to be used for the detection of A β . In this thesis we, therefore, developed a new monoclonal antibody (mAb) against the N-terminal end of A β (VU- α -A β 17) and VU- α -A β 17 was used to stain brain slices of several AD patients. Additionally, VU- α -A β 17 was compared with other mAbs.

Figure 2



AD starts and should be identified before of occurrence of full-blown dementia (as for other dementing conditions)⁵⁸; VD=vascular dementia, FTLN=frontotemporal lobar degeneration, LBD=Lewy body disease, MCI=mild cognitive patients.

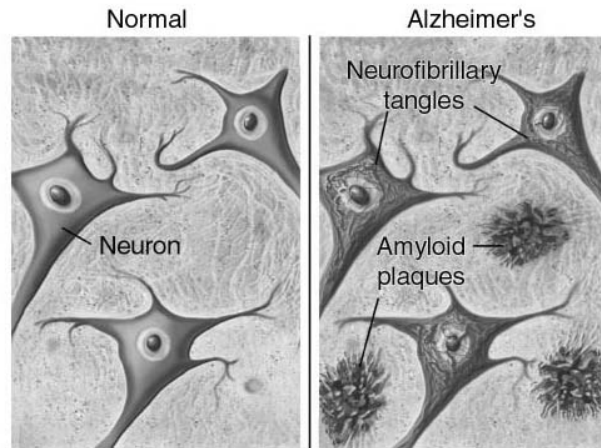
Measuring A β 40, A β 42, Tau and P-Tau in CSF

Since biochemical changes in the brain are thought to be reflected in the CSF, the levels of A β 40, A β 42, Tau, P-Tau and other related factors (or associated factors) have been investigated and measured extensively in CSF. A pronounced increase in CSF levels of Tau and P-Tau proteins, and a decrease in the CSF level of A β 42, have been found in AD patients compared to control subjects^{7, 59, 60}. For this reason measuring A β 42, Tau and P-Tau in CSF has gained wide recognition, not only to discriminate AD patients from controls or patients with other types of dementia⁶¹⁻⁶³, but also to detect incipient AD in MCI patients^{64, 65}.

These biomarkers have thus earned their place in the diagnostic repertoire. Less studies have been performed with A β 40, since similar levels of CSF A β 40 were found in AD^{61, 62, 66-73} compared to controls. However, it seems to be that A β 40 has additional value in differential diagnosis^{61, 72}. To confirm these findings, we investigated this in a large population of patients. Since these biomarkers can be measured in CSF and have been shown to be useful in clinical practice, the number of laboratories measuring these peptides has increased substantially. This resulted not only in publications reporting high inter-assay

variation of measurements⁷⁴, but also in different reference values between centres^{62, 75-80}. These variations are partly due to pre-analytical and analytical factors^{74, 81-84}. To decrease these variations we organized the first world-wide quality control for CSF biomarkers in AD and we studied the intra-centre variations during time.

Figure 3



Neuropathological changes in AD are characterized by senile plaques and neurofibrillary tangles^{27, 28, 85}.

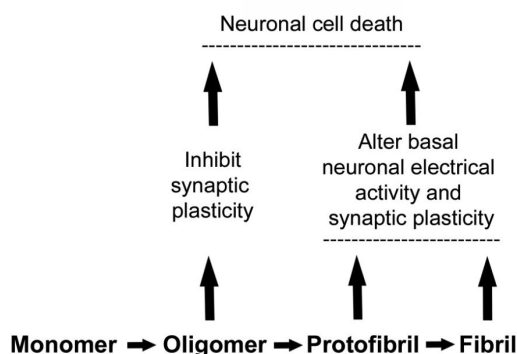
Table 1

Neuropathological assessment of the likelihood that AD accounts for a dementia should be judged as follows^{56, 57}

1. Likelihood is high when the post-mortem brain shows both neuritic plaques and neurofibrillary tangles in the neocortex (i.e. frequent neuritic plaques score according to CERAD and stage V/VI according to Braak).
2. Likelihood is intermediate when there are moderate neocortical neuritic plaques and neurofibrillary tangles in limbic regions (i.e. CERAD moderate, and Braak stage III/IV).
3. Likelihood is low when there are neuritic plaques and neurofibrillary tangles in a more limited distribution and/or severity (i.e. CERAD infrequent, and Braak stage I/II).

ELISAs (Enzyme-Linked Immuno Sorbent Assay; one of the systems used to measure peptides and proteins) rely on the specificity of the incorporated (m)Abs, on the stability of the peptide that has to be measured, on matrix effects of the specimen, and the availability of well-characterized and stable standard preparations⁸⁶. Robust measurement systems would be very helpful to decrease the variability⁸⁶. To reach this goal an extensive screening, purification and characterization of mAbs used in an ELISA is needed, together with a complete validation of the ELISA⁸⁶. We tried to build a robust system and to reach this goal we produced a new mAb against the C-terminus of A β 1-40 and built this in an ELISA system.

Figure 4



*Aβ toxicity is likely to be mediated by multiple different Aβ assembly forms*³².

Since the publication of the NINCDS–ADRDA criteria (1984), considerable progress has been made toward understanding different aspects of AD. One of the important features is research in CSF levels of Aβ₄₂, Tau and P-Tau. Changes in concentrations of these peptides have shown to be able to characterize AD more in phenotypical way⁷. As a result, many studies have specifically addressed the value of cerebrospinal fluid biomarkers in the clinic and proposed new clinical criteria for AD⁵⁸. In chapter 5 we tested in a practical way the newly proposed clinical criteria.

1.2

Aims of the thesis

The objectives of this thesis were:

1. To produce and characterize mAbs against Aβ.
2. To validate measurement systems for AD biomarkers in CSF.
3. To study the implementation of AD biomarker measurement systems in the clinic.

In **chapter 2**, we describe the production and the characterization of two mAbs (VU-α-Aβ₁₋₁₇ and VU-α-Aβ₄₀). VU-α-Aβ₁₋₁₇ has been pathologically validated. VU-α-Aβ₄₀ has been built in an ELISA system that was validated analytically and clinically.

In **chapter 3**, we assessed the influence of assay variation on levels of Tau and P-Tau in CSF. Subsequently, we addressed the internal validation during six years experience of CSF Aβ₄₂, Tau and P-Tau measurements. In line with this, we set out the first world-wide quality control for these three CSF biomarkers.

In **chapter 4**, we investigated the value of CSF Aβ₄₀ in differential diagnosis. Additionally, we studied the usefulness of SAP for early diagnosis and the use of plasma biomarkers for detection of AD. Finally, in **chapter 5**, we assessed the value of the newly proposed research criteria for AD⁵⁸.