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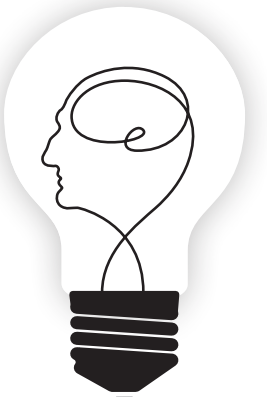
# Chapter 1

## General Introduction



Worldwide millions of elderly and their relatives suffer from the devastating effects of dementia, with Alzheimer's disease (AD) as its most common cause.[1] Age is its main risk factor, and as the worldwide average life expectancy is increasing, the incidence of dementia is expected to rise. Clinically, AD is characterized by a progressive loss of episodic memory in combination with impairment in other cognitive domains and behavioral changes. Eventually severe loss of cognition leaves the patient completely dependent, with death occurring on average nine years after manifestation of the first symptoms. As a result, AD represents an important socio-economic and public health concern.[2] Unfortunately, an effective therapy is currently not available, which is due to our incomplete understanding of the pathogenesis of AD. Our inability to detect the disease at its early stages prevents furthering our insight into the pathogenesis on the one hand, and on the other it prevents us to detect the disease at a stage early enough to expect a beneficial effect of treatment. Nowadays, a definitive diagnose of AD still requires an autopsy. Therefore, novel diagnostic methods are warranted that are capable of obtaining a definite diagnosis during life and preferably as early in the disease process as possible.

**Pathogenesis**

Microscopic examination of AD brain tissue typically reveals the presence of both extracellular deposits of fibrillar amyloid-β peptides and intracellular neurofibrillary tangles in the medial temporal lobe structures and cortical areas of the brain in combination with a deterioration of neurons and synapses. (**Figure 1.1**) These changes are assumed to play a vital role in the pathogenesis of the disease.

**Neurofibrillary tangles**

Neurofibrillary tangles (NFTs) consist of abnormally phosphorylated tau proteins that predominantly accumulate in neurons. Normally, tau proteins promote the assembly of microtubule and their stability, and as such they are involved in structural and regulatory functions of the cytoskeleton.[3,4] However, when tau becomes hyperphosphorylated it exerts the exact opposite effect, leading to the disassembly of the same microtubules. The resulting

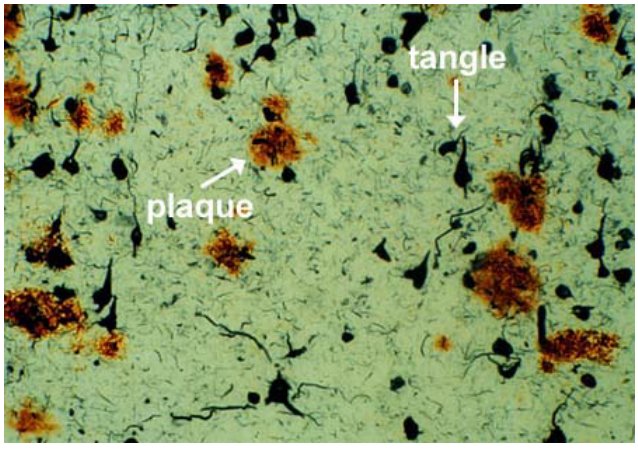



**Figure 1.1 Neuropathological characteristics of Alzheimer's disease**

AD is neuropathologically hallmarked by the cortical deposition of amyloid-β peptides into amyloid or senile plaques that are surrounded by neurofibrillary tangles present in the axons and neuronal cell bodies.

loss of neuronal structure impairs axonal transport, thereby disturbing proper synaptic and neuronal signaling.[3] In addition, as hyperphosphorylated tau tends to aggregate into insoluble filaments, it becomes sequestered into NFTs deposited in neurons and neuronal processes, also known as neuropil threads. These NFTs further disrupt normal neuronal function and eventually lead to neuronal death.

Although tau-pathology is observed within several neurodegenerative disorders[3], its characteristic topographical spreading observed in AD led to the description of a histological staging of the disease by Braak and Braak.[5] This AD staging sequentially describes the involvement of the transentorhinal and enthorhinal cortex (stage I and II), the hippocampus (stage III and IV), and finally the isocortex (stage V and VI). Of all pathological hallmarks, tau pathology has shown the best clinicopathological correlation in AD patients.[6] However, the pathophysiological role of hyperphosphorylation and tangle formation is still incompletely understood.

**Deposition of amyloid-β**

Amyloid-β (Aβ) peptide is generated by proteolytic cleavage of a transmembrane protein, amyloid precursor protein (APP), by β- and γ-secretase to form predominantly $Aβ_{1-40}$ or $Aβ_{1-42}$. Under normal conditions, cerebral Aβ is either degraded or cleared from the brain by a balanced process of influx and efflux across the blood-brain barrier (BBB). According to the amyloid cascade hypothesis, AD is initiated by an imbalance in Aβ production and clearance.[7,8] This is supported by observations in familial AD, accountable for < 5% of all cases, in which mutations in the genes encoding for APP or parts of the secretase complexes cause Aβ overproduction. In the majority of cases, so called sporadic AD, a failure of Aβ clearance is thought to gradually increase cerebral levels of Aβ over time.

Its fibrilogenic nature causes high local concentrations of $Aβ_{1-42}$ to aggregate, first into soluble oligomers. These eventually cluster into larger insoluble Aβ fibrils that allow the formation of β-sheet structures characteristic for amyloid. Serving as a seeding point this triggers the misfolding of other Aβ species, including the more soluble $Aβ_{1-40}$.

Histologically, Aβ deposits in the brain parenchyma can be classified into two distinct types: non-neuritic (diffuse) or neuritic (senile) Aβ plaques. Diffuse plaques typically only show up on Aβ immunohistochemistry, whereas no amyloid structure is detected by Congo red of Thioflavin staining.[9] In contrast, senile plaques consist of an amyloid core of predominantly $Aβ_{1-42}$ surrounded by a neuritic corona baring degenerative synaptic endings with hyperphosphorylated tau, together with activated astrocytes and microglia.[9]

In addition, deposits of amyloid, mainly $Aβ_{1-40}$, can also be found in the cerebral vessel wall where it leads to a loss of structure and rigidity. This so-called cerebral amyloid angiopathy (CAA) can occur by itself without any parenchymal involvement, and is considered a major cause of cerebral microbleeds, hemorrhages and cognitive loss. In 90% of all cases, however, AD and CAA are concomitant.[10]

Similar to tau, the presence of Aβ deposits can be staged based on the spread of cortical involvement: (A) only basal portions of the isocortex; (B) throughout the isocortex except the primary cortices and with the hippocampus mildly affected; (C) the entire isocortex.[5] A correlation between cerebral Aβ accumulation and cognitive status has been shown, albeit less significant

in comparison to tau. However, the soluble pool of (oligomeric) Aβ are thought to contribute more to Aβ-mediated pathology than the histological insoluble Aβ deposits; and the occurrence of oligomers has been shown to correlate better with the cognitive status.[11]

**Hypothetical models**

Based on observations in large-scale post-mortem studies in the general aging population, the AD induced neurodegeneration is estimated to start two decades before clinical onset and is thought to have reached a plateau at the time of clinical presentation.[12,13] (**Figure 1.2**) The exact underlying pathogenesis responsible for the Aβ imbalance, the hyperphosphorylation of tau and their intimate association remains one of the major unresolved questions regarding AD. What triggers their formation and is their presence a cause or only a consequence of the disease? Besides the aforementioned amyloid cascade hypothesis, some claim that the dyshomeostasis of cerebral iron plays an intricate and crucial role. Increased cortical accumulation of iron is often found within the premises of the amyloid plaques. As its presence is known to lead to formation of reactive oxygen species that induce neurotoxicity, this could eventually lead to neuronal cell death and loss of cognitive function. Interestingly, not only APP and Aβ but also tau is involved in the chelation and transport of cerebral iron. Others go one step further: as iron is essential for the enzymes responsible the maintenance of myelin structure and integrity, they have suggested that a mismatch in de- and remyelination might change iron homeostasis leading to increased iron deposition, which, subsequently, could lead to increased production of Aβ and tau.[14] However, as yet no clear single explanation regarding the pathogenesis of AD has been found.

**Diagnosis**

At present, the definite diagnosis of AD can only be made based on microscopical detection of Aβ and NFTs in brain tissue, which in general happens at autopsy. Clinically, the diagnosis "probable AD" can be made at best based on criteria set by the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) and the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association).[15] These criteria require a detailed (hetero) anamnestic assessment of the type and course of symptoms, while other somatic causes of dementia, such as cerebral infarcts, neoplasms or hypothyroidism, have to be excluded. Clinically, AD is characterized by progressive loss of memory and gradual decline of other cognitive domains affecting social and occupational functioning. Typically, the symptoms start with isolated short-term memory loss. Over time, long-term memory becomes involved too. Characteristically for AD, long-term memory loss occurs in a retrograde manner with early memories being preserved longer than recent ones. Apart from memory loss, apathy occurs as well as cognitive loss in other domains resulting in difficulties in speech (aphasia), practical skills (apraxia), recognition (agnosia) and/or executive functions. As a result, patients become restricted in their activities of daily living (ADL) and lose their ability to safely support themselves, which eventually leaves them fully dependent on family or institutionalized care.

The criteria developed by NINCDS-ARDA provide a clinical diagnostic tool with a relatively high sensitivity and specificity (>80%) to distinguish AD patients from elderly people without dementia.

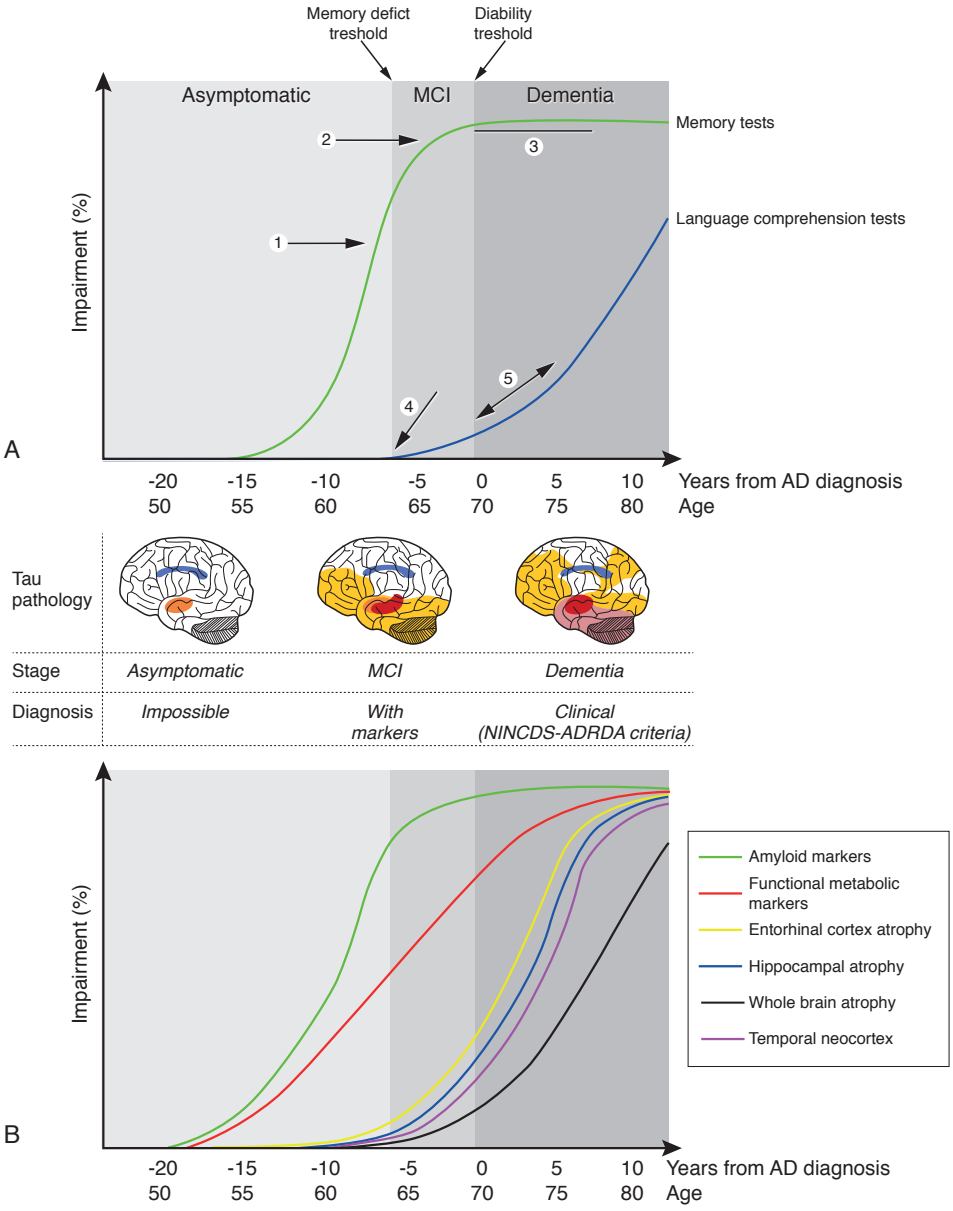



**Figure 1.2 A theoretical model of the natural progression of cognitive and biological markers of Alzheimer's disease**
*(reprinted and adapted with permission[13])*
(**A**) Regarding cognition the memory tests are among the first to change (1). As they quickly reach their maximal level of impairment (2), they are useful for diagnosis at MCI, but are less adequate to track disease progression (3). Later in the disease, the language comprehension starts to change with mild or no impairment during MCI (4), and a steep increase during dementia (5). (**B**) Amyloid markers, e.g. [$A\beta_{42}$] in the cerebral spine fluid and amyloid PET, represent the earliest detectable changes in AD, but plateau at the MCI stage. Metabolic and functional biomarkers, like $^{18}$F-FDG PET and fMRI, are abnormal at the MCI stage, and continue to change far into the dementia stage. Cerebral atrophy appears later and follows a temporal pattern mirroring the deposition of tau pathology.

Using these criteria, the distinction between AD and other neurodegenerative dementias however is less accurate (23–88%).[16] In an earlier stage of the disease when objective memory complaints are present but daily functioning remains normal and other cognitive domains are still intact, subjects qualify for the diagnosis mild cognitive impairment (MCI). However, not all MCI subjects are believed to have AD and to develop dementia later in life. (**Figure 1.2**)

**The role of imaging biomarkers in AD**

Although its precise etiology remains unknown, the onset of AD is assumed to start two decades before the induced neuronal damage has reached a sufficient level to lead to noticeable clinical symptoms.[12,13] Our incomplete understanding of the pathophysiology of AD is partly to be blamed on our inability to detect the early phases of the disease reliably *in vivo*, and our lack of understanding of the pathophysiology of AD is a major hurdle in developing effective treatment. Apart from being instrumental to the development of AD treatment, having early markers of the disease is also relevant since it would allow distinguishing among elderly individuals with memory loss between those in whom this symptom is an early manifestation of AD and progression to dementia can be expected from those in whom functional loss is not expected to spread to other cognitive domains or when other diseases than AD play a role. Furthermore, once effective treatment will be available, it would be useful to detect even earlier, preferably presymptomatic, stages of the disease, in order to have a wider time window for treatment. Finally, a test that would allow for detection of the early stages of the disease would also be a very useful tool to assess the efficacy of candidate treatment in trials.

In general such a diagnostic method or so-called biomarker refers to an objectively measurable physiologic, biochemical, or anatomic parameter that represents a (patho)physiologic process or a therapeutic response.[17] In search of a biomarker for AD major efforts have been made to develop methods that allow non-invasive *in vivo* detection of AD-specific changes using neuroimaging techniques.

**Structural, functional and metabolic neuroimaging techniques**

Initially CT and MRI scans of the brain were just used in patients with dementia to detect possible treatable causes of the symptoms, such as subdural hematomas and meningiomas, but not for the detection of the more prevalent neurodegenerative disorders in such patients. Later, these techniques were used to detect radiological manifestations of these neurodegenerative diseases. In AD patients structural MRI studies revealed specific patterns of regional cerebral atrophy. Severity of atrophy was observed to correlate well with loss of cognitive function as well as with post-mortem Braak staging. Of all putative biomarkers, volumetric measures of hippocampal and medial temporal lobe atrophy showed the best correlation with the severity of clinical symptoms. According to the dynamic biomarker model (**Figure 1.2**), however, brain atrophy is a late stage event, and therefore structural MRI may not be the method of choice for early or pre-symptomatic diagnosis.

In addition to brain atrophy, the feasibility to detect the histological hallmarks of AD using MRI has also been explored. In general these efforts have focused on detecting individual amyloid plaques. Recently it was demonstrated that increased iron accumulation in amyloid plaques combined with the aggregated protein itself induce a magnetic susceptibility effect, visible as

hypointense foci on $T_2$*-weighted or susceptibility-weighted (SW) MRI in the cerebral cortex of transgenic AD mouse models and in human post-mortem brain slices.[18-20] The high magnetic field strengths needed to obtain these results only recently became available for *in vivo* human use. These high field whole body human MRI systems (≥7 Tesla) may offer new possibilities to specifically detect these neuropathological hallmarks of AD; perhaps even at an earlier stage than the traditional MRI biomarker of brain atrophy.

Besides these conventional MRI techniques the versatile character of MRI offers additional possibilities to study changes caused by AD, as has been extensively reviewed elswhere.[21-23] In short, functional MRI (fMRI) reflects brain activation when a specific task is performed, and in subjects at risk to develop AD fMRI discovered areas of hyperactivation following specific tasks. Using task-free resting state fMRI a decrease in activity of the brain's default functional network was seen in AD subjects. Additionally, specific regions of cerebral hypoperfusion were found in relation to AD using non-invasive arterial spin labeling MR perfusion techniques. Whereas microstructural changes due to AD may not be directly visible, their effect on bulk MR signal could allow detection by quantitative MRI techniques, as shown by initial studies using magnetic transfer imaging (MTI), diffusion tensor imaging (DTI) and MR relaxometry. Finally, changes in brain metabolites have been observed in AD subjects as MR spectroscopy revealed a consistent decrease in *myo*-inositol combined with elevated levels of *N*-acetylaspartate (NAA). Although these MRI techniques just recently gained much interest, currently none has been characterized and validated well enough yet to be included as a neuroimaging biomarker for AD.

**Molecular imaging techniques**

Direct visualization of the histological hallmarks of AD has been attempted by the application of molecular imaging strategies. These strategies aim for the *in vivo* imaging of specific molecular or cellular signatures of the disease with the aid of targeted contrast agents. Thus far two molecular imaging strategies have been developed for AD: detection of glucose hypometabolism using $^{18}$F- fluorodeoxyglucose (FDG) PET, and visualization of cerebral amyloid load by radioactive PET ligands. FDG-PET reflects brain metabolism, which in general is a function of neuronal or synaptic activity. Typically for AD is a decrease in FDG uptake in the lateral temporal-parietal, posterior cingulate and precuneus regions that indicates impaired neuronal activity in these areas.[12] Even when corrected for brain atrophy these patterns of glucose hypometabolism were seen. Despite the fact that these patterns of glucose hypometabolism occur prior to the development brain atrophy detectable on neuroimaging, following the amyloid cascade hypothesis their specificity is limited and they are considered a relatively late phenomenon. Therefore major efforts have been made to develop imaging agents that specifically bind to AD's neuropathological hallmarks. Based on known histological amyloid dyes, like Congo Red and Thioflavin, various radioactive contrast agents have been synthetized for the *in vivo* detection of cerebral amyloid by either PET or SPECT imaging. The initial *in vivo* breakthrough came with the development of Pittsburgh Compound B (PiB), a neutral $^{11}$C derivative of Thioflavin.[24] Despite many *in vivo* human studies its broad applicability is hampered by its short radioactive half-life (<20min), requiring the availability of a cyclotron and therefore its application is mainly limited to research. Another limitation of PiB PET imaging is the inability to distinguish between Aβ

deposits in parenchymal plaques and in the vessel wall (as in CAA). Thus, Aβ targeting imaging techniques are needed that can be performed on widespread neuroimaging platforms and that are able to discriminate between the different types of Aβ deposits.

**Aim of this thesis**

This thesis is aimed at the development of innovative diagnostic imaging techniques to detect the histological signatures of AD using emerging ultra-high field MRI technologies and molecular imaging strategies. The work in this thesis comprises two complementary parts.

**Part One** aims at developing novel MRI techniques for the *in vivo* detection of cortical changes in AD exploiting innovative ultra-high field MRI technology (7 Tesla). Initially, we focused on developing techniques optimized for tissue iron detection at ultra-high magnetic field. Then we strived to obtain a better understanding of the source of native MRI contrast changes specific for AD neuropathology by studying post-mortem AD tissue at experimental MRI systems with optimized MR techniques and by systematically comparing MRI and histological data.

The main objective of **Part Two** is the detection of Aβ deposits using targeted contrast agents. Firstly, we aimed at developing imaging probes that allow distinguishing specific types of Aβ deposition, e.g. vascular versus parenchymal, based on llama antibody fragments. Secondly, we aimed at improving small amyloid binding molecules to serve as *in vivo* imaging probes for $^{19}$F MRI or other imaging modalities.

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