Dissertation

Magnetization Transfer Imaging: a non-conventional MRI technique for the longitudinal assessment of Alzheimer's disease

Submitted by

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Declaration

I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the guidelines of „Good Scientific Practice“.

Parts of this dissertation have already been published (own work) and may therefore resemble in content and syntax.

Graz, 16.08.2021 Isabella Giusy Colonna

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Abbreviations

AD	Alzheimer´s disease
Aβ	Amyloid-β
APP	Amyloid-β precursor protein
CERAD	Consortium to Establish a Registry for Alzheimer´s disease
CNS	Central nervous system
CSF	Cerebrospinal fluid
DTI	Diffusion Tensor Imaging
FA	Fractional anisotropy
FLAIR	Fluid attenuated inversion recovery
GM	Grey matter
HC	Healthy controls
MCI	Mild Cognitive Impairment
MD	Mean diffusivity
MMSE	Mini Mental State Examination
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MTI	Magnetization Transfer Imaging
MTR	Magnetization Transfer Ratio
NAWM	Normal appearing white matter
PET	Positron emission tomography
QSM	Quantitative susceptibility mapping
ROI	Region of interest
SPSS	Statistical Package for the Social Sciences
WB	Whole brain
WM	White matter
WMH	White matter hyperintensities

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Abstract in German (Zusammenfassung)

Hintergrund

Magnetisierung Transfer ist eine neuartige quantitative Magnetresonanztomografie (MRT) Methode, welche mikrostrukturelle Hirngewebsveränderungen erfassen kann. Ziel unserer Arbeit war es die Magnetisierungstransferratio (MTR) in grauer und weißer Substanz bei Personen mit der Alzheimer Krankheit und gesunden Kontrollpersonen zu vergleichen, den Zusammenhang zwischen MTR und kognitiver Leistungsfähigkeit sowie die Assoziation zwischen longitudinalen MTR Veränderungen und kognitiven Veränderungen zu untersuchen.

Methoden

In dieser prospektiven Studie wurden Patienten mit der Alzheimer Krankheit und gesunde Kontrollpersonen mittels 3 Tesla MRT untersucht. Die MTR Werte wurden im Kortex, in den„Alzheimer-signature regions“, in der normal erscheinenden weißen Substanz des Gehirns und in Marklagerhyperintensitäten untersucht. Die kognitive Funktion wurde bei Patienten mit der Alzheimer Krankheit mit der „Mini Mental State Examination“ (MMSE) und mit den neuropsychologischen Testungen des „Consortium to Establish a Registry for Alzheimer´s disease“ (CERAD) erfasst. Die statistische Analyse wurde mit SPSS 25 durchgeführt.

Ergebnisse

Siebenundsiebzig Patienten mit Alzheimer Krankheit (Durchschnittsalter ± SD : 72 ± 8 Jahre; 47 Frauen) und 77 gesunde alters-gematschte Kontrollpersonen (Durchschnittsalter ± SD : 72 ± 8 Jahre; 44 Frauen) wurden in die Studie inkludiert. Die MTR Werte waren bei Patienten mit der Alzheimer Krankheit in den untersuchten Regionen niedriger als bei den Kontrollpersonen. Es bestand eine indirekte Assoziation zwischen den MTR Werten im globalen Kortex (OR = 0.47; 95% CI: 0.22, 0.97; P = 0.04), in den „Alzheimer Signature Regionen“ (OR= 0.31; 95% CI: 0.14, 0.67; P = 0.003), in der normal erscheinenden weißen Substanz (OR = 0.59; 95% CI: 0.39, 0.88; P = 0.01) und in den Marklagerhyperintensitäten (OR = 0.18; 95% CI: 0.09, 0.33; P < 0.001) mit der Alzheimer Diagnose. Niedrigere MTR Werte in der grauen Substanz waren mit verminderter globaler Kognition, Sprachfunktion und konstruktiver Praxis assoziiert. Diese Zusammenhänge waren unabhängig von demographischen Daten, Läsionen der weißen

Substanz und normalisiertem regionalem Hirnvolumen. Siebenundvierzig Patienten mit Alzheimer Krankheit hatten eine Follow-up Untersuchung nach 1.06 (± 0.24) Jahren, wobei eine signifikante Reduktion der MTRs im globalen Kortex beobachtet wurde. Die longitudinalen MTR Veränderungen standen nicht mit Veränderungen der kognitiven Funktionen in Zusammenhang.

Schlussfolgerungen

Die Alzheimer Krankheit geht mit einer Reduktion der MTR in der grauen sowie auch weißen Substanz einher. Niedrigere MTR Werte im Kortex und in den „Alzheimer Signature Regionen“ sind mit kognitiver Beeinträchtigung bei AD-Patienten unabhängig von demographischen Daten, Läsionen der weißen Substanz und Atrophie, assoziiert. Bei Patienten mit Alzheimer Krankheit wurde zwar eine signifikante jährliche Reduktion der MTR Werte im Kortex beobachtet, jedoch ohne Korrelation mit dem kognitiven Status.

Abstract in English

Background

Magnetization Transfer Imaging can detect microstructural brain tissue changes that are not assessable by conventional magnetic resonance imaging and may be helpful in Alzheimer´s disease (AD) diagnosis. The aim of the present thesis was to compare magnetization transfer ratio (MTR) measures in global and regional grey and white matter between patients with Alzheimer´s disease and healthy control participants, to analyze the association between MTRs and cognition in AD, and to investigate the MTR changes and their association with cognitive decline after a follow-up time in AD.

Material and methods

In this prospective study, patients with Alzheimer disease and a group of age-matched healthy controls underwent clinical examinations and 3T MRI. MTRs were determined in the cortex, in six areas that have been seen to be particularly vulnerable to AD, namely the "AD-signature regions", in the normal-appearing white matter, and in the white matter hyperintensities. The cognitive function was assessed in AD patients with Mini Mental State Examination and the Consortium to Establish a Registry for Alzheimer Disease test battery. Statistical analysis were performed with SPSS 25.

Results

Seventy-seven patients with AD (mean age ± SD, 72 ± 8 years; 47 female) and seventy-seven age-matched healthy control participants (mean age ± SD, 72 ± 8 years; 44 female) were assessed. MTR measures were reduced in patients with AD than in healthy control individuals in all considered regions. When adjusting for brain volume and extent of white matter hyperintensities, AD diagnosis was significantly associated with lower MTRs in global cortex (OR = 0.47; 95% CI: 0.22, 0.97; P = 0.04), in AD signature regions (OR= 0.31; 95% CI: 0.14, 0.67; P = 0.003), in normal-appearing white matter (OR = 0.59; 95% CI: 0.39, 0.88; P = 0.01) and in white matter hyperintensities (OR = 0.18; 95% CI: 0.09, 0.33; P < 0.001). Further, lower grey matter MTRs were related to poorer global cognition, language function, and constructional praxis in AD. Forty-seven patients of the AD cohort underwent a subsequent

clinical, neuropsychological and neuroimaging assessment after a follow-up time (mean years ± SD = 1.06 years ± 0.24). In the longitudinal analysis, a significant MTR decline over time was seen only in the global cortex (annualized MTR change: median = -3.86, $P < 0.001$); however, it not related to cognitive decline.

Conclusions

Alzheimer disease is associated with lower MTR values in grey and white matter regions of the brain. Reduced MTRs in the global cortex and AD-signature regions contribute to cognitive decline in AD, independent of brain volume and extent of white matter damage. Only cortical MTRs declined significantly over the follow-up time in AD.

Disclosures

Part of this thesis has been published in the following article:

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I.C., S.R. and R.S. devised the project and the main conceptual ideas; I.C. performed the statistical analysis and wrote the manuscript with input from all the authors; L.P. worked out the technical details and processed the MRI data; E.H. supervised the statistical analysis; R.S., S.R., P.S., C.E., M.K. and A.D. contributed to the interpretation of the findings. All the authors provided critical feedback and helped shape the research, analysis and manuscript.

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Introduction

General background

Dementia is an acquired progressive cognitive impairment that affects the activities of daily life and represents a relevant cause of dependence, disability and mortality (1). It has a significant impact on the quality of life of patients and their caregivers, in addition to necessitating costly medical, social and informal care (2).

Alzheimer's disease (AD) is the most common cause of dementia and has been recognized as a public health priority by the World Health Organization (1).

Since AD-related neuropathological changes may occur several years before the onset of symptoms, in the last decades efforts have been made to define reliable biomarkers that may enable an earlier and more precise diagnosis than the clinical assessment (3).

Currently, no disease-modifying therapy for AD is available, and more than two hundred therapeutic agents have been evaluated in failed or abandoned trials (4). One possible explanation of these failures is that these potential treatments have been tested too late in the pathophysiological course of AD (4). Thus, an earlier identification of AD pathological changes through reliable biomarkers might be of utmost importance for future drug development (4).

Magnetic resonance imaging (MRI) is a non-invasive and accessible method that allows to study *in vivo* brain alterations typical of aging, as well as of neurodegenerative diseases such as AD. While structural MRI is suitable to study macroscopic brain changes, new MRI neuroimaging methods, such as diffusion weighted imaging and magnetization transfer imaging (MTI), are useful for the detection of alterations of the brain's microstructure, which may occur many years before the disease´s onset (5).

In the present study, we used MTI to identify microstructural brain alterations in the grey and white matter of a large cohort of patients with AD, and to investigate their associations with cognitive impairment, independent of macroscopic brain changes.

Further, we performed a longitudinal analysis with the aim to investigate the annualized changes of MTI metrics and their relationship with cognitive decline over a follow-up time in AD.

Alzheimer's disease

This chapter will give a short overview on Alzheimer's disease

Epidemiology

Worldwide, over 46 million people live with dementia; this prevalence is estimated to increase to 131.5 million by 2050 (2). Every year there are nearly 10 millions new cases in the world. The increasing burden of dementia is related to higher life expectancy, with prevalence growing most rapidly in the population aged 60 years or older (6). For this reason, in the last years, increasing attention is given to dementia from governments and policy-makers (7).

Recently, some studies observed a slow decrease in the incidence of dementia in western countries; the reasons of this decline are unclear but might be related to the improvement in the control of cardiovascular risk factors (1,7). In the next decades, the prevalence of dementia is expected to rise in low and middle income countries, where the incidence of diabetes, hyperlipidemia, hypertension and cardiovascular disease is increasing (1).

Alzheimer's disease is the most common cause of dementia, representing 60-80% of dementia cases (8). In Europe, the prevalence of AD has been estimated at 5.05%, with an incidence of 11.08 per 1000 person-year. Higher AD prevalence has been seen in women than in men (9).

Risk factors

The majority (>95%) of AD cases occur on an apparently sporadic basis, have a late onset (10) and are likely to be driven by a complex interaction between genetic and environmental factors (1). Twelve potentially preventable risk factors in early life (education), midlife (hypertension, obesity, hearing loss, traumatic brain injury, and alcohol misuse) and later life (smoking, depression, physical inactivity, social isolation, diabetes, and air pollution) have recently been associated with increased dementia risk; preventative interventions aimed to the control of these risk factors might avoid or delay up to 40% of dementias (11).

Age is the most important non-modifiable risk factor for AD (12). Female sex is associated with higher risk for AD as well; however, this greater risk has been attributed to women's greater longevity (12).

According to Ballard et al. (13), around 70% of the amount of risk for AD is attributable to genetics. Several genetic polymorphisms have been associated with higher risk for AD (13). Among them, the presence of Apolipoprotein E- ε4 allele has been considered the main genetic determinant of AD risk (14).

Apolipoprotein E, whose gene is located on chromosome 19q13.2, is responsible for the regulation of lipoproteins and is involved in the neuroplasticity as well as in the cholesterol transport and inflammation in the central nervous system (15). Moreover, Apolipoprotein E influences the metabolism of amyloid β, by regulating its clearance and aggregation, and by interacting with receptors such as low density lipoprotein receptor-related protein 1(15). Neuroimaging studies observed that among a large sample of cognitively normal individuals, aged from 45 to 88 years old, the subjects who were carriers for Apolipoprotein E- ε4 allele showed increased mean cortical binding potential for Pittsburgh Compound-B at the amyloid-PET and lower levels of Amyloid β – 42 in the cerebrospinal fluid, compared to non-carriers individuals (16). Similarly, Apolipoprotein E- ε4 allele has been associated with increased tau pathology (17). It has been shown that the presence of one Apolipoprotein E- ε4 allele is related to a threefold higher risk for AD, while two Apolipoprotein E- ε4 alleles increase the AD risk by 12 fold (15). By contrast, individuals presenting Apolipoprotein E- ε2 allele have reduced risk to develop AD (15).

In addition to Apolipoprotein E, genome-wide association studies detected other less frequent and less strongly associated genetic risk factors, involving phosphatidylinositol-binding clathrin assembly protein (*PICALM*), *CD33*, triggering receptor expressed on myeloid cells 2 (*TREM2*), ATP-binding cassette transporter *ABCA7*, clusterin (*CLU*) and complement receptor type 1 (*CR1*)(10).

Only few patients (<1%) are affected by familiar forms of Alzheimer disease, which typically show an early onset of the symptoms (1) and are caused by mutations of the genes encoding amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) and 2 (*PSEN2)* (10,13).

The gene for APP, located at the chromosome 21, encodes a membrane glycoprotein, whose proteolysis determines the production of different peptides, including Amyloid β (18). Mutations in this gene are autosomal dominant and are detected in the 16% of patients with familiar early onset AD (18).

PSEN1 and PSEN2 genes, located at the chromosome 14 and 1 respectively, encode two proteins, which are present in multiple tissues, including the brain, and are particularly elevated in the hippocampus and in the cerebellum (18).

Pathogenesis

Although many research efforts in the last decades, the pathogenesis underlying AD is still unclear. The majority of proposed pathogenic mechanisms arise from two hypotheses: the amyloid cascade hypothesis and the tau hyperphosphorylation hypothesis (19).

Amyloid hypothesis

The central role of amyloid in AD pathogenesis is supported by several genetic and biomarkers studies in both familial and sporadic AD (20). Amyloid β (Aβ) is a transmembrane protein derived from the hydrolysis of the Aβ precursor protein (APP) (20). Although its biological function remains unclear, this protein is widely expressed in several tissues, with a high concentration in the brain (21).

Three pathways for the hydrolysis of APP have been identified: the non-amyloidogenic, the amyloidogenic and the η-secretase pathway (21).

In the amyloidogenic pathway, the γ-secretase is responsible for the production of two monomer species of Aβ peptides: Aβ40 and Aβ42. The Aβ40 peptides are involved in the generation of around 90% of all Aβ fragments; Aβ42 peptides are more prone to form oligomers and fibrils (21) and have stronger neurotoxicity than Aβ40 (19). Aβ peptides accumulate in plaques and induce mitochondrial damage, unstable homeostasis, synaptic dysfunction and inflammation (19).

In the past two decades, the amyloid hypothesis has become the most widely accepted model for pathophysiology of AD, and it has guided the development of potential treatments (22). Although several studies on mouse models indicated that anti-amyloid immunotherapy could prevent amyloid plaque formation and improve mouse behavior (23,24), the translation of these animal results into human trials has been a failure. The lack of success of these promising therapy for AD is increasingly questioning the amyloid hypothesis.

Post-mortem studies reported that about 40% of non-demented individuals presented brain amyloid pathology (25). Similarly, neuroimaging studies observed that 47% of cognitively normal subjects might present amyloid positive positron-emission tomography scans (26). The presence of amyloid pathology in cognitively intact individuals remains still unclear (26). However, it has been reported a correlation between brain amyloid pathology and higher likelihood of development of mild cognitive impairment or AD, as well as poorer performance on the MMSE and presence of subjective cognitive decline (26).

Tau hyperphosphorylation hypothesis

Tau is a protein that is mainly found in the axons of the brain (27); its role is to stabilize the microtubule and synaptic structure, to regulate cytoplasmatic transport as well as neuronal signaling (19), to maintain the neuronal polarity and to enhance the axonal elongation (27). Six isoforms of tau proteins, produced through alternative splicing from single gene located at the chromosome 17, have been found in the adult brain (28).

Abnormal alterations and aggregation of tau protein have been associated with AD and other neurodegenerative disorders called "tauopathies", such as frontotemporal dementia or progressive supranuclear palsy (29).

Phosphorylation has been found to be the most frequent modification of tau protein (30). Tau phosphorylation is not only involved in pathological changes, but it also serves to regulate tau in a physiological sense (27).

Under normal conditions, tau isolated from human brain tissue has been shown to carry few sites for phosphorylation (30) and to negatively regulate the interaction between tau and microtubules (19).

In pathological states, the phosphorylation of tau is saturated (19). Hyperphosphorylated tau has been reported in AD and might determine structural changes of tau protein and loss of tubulin polymerization capacity, determining impaired microtubule functioning and accumulation of neurofibrillary tangles (19).

Several protein kinase are responsible for tau phosphorylation, such as glycogen synthase kinase-3β, cyclin dependent kinase 5, or microtubule affinity regulatory kinases (31). Tau phosphorylation may be enhanced by inflammation, as suggested by preclinical studies, which

reported an association between over-expression of interleukin 1β and increased phosphorylation of tau protein in transgenic murine model of AD (32).

Moreover, tau phosphorylation might be induced also by amyloid β, as reported in vitro and in vivo models as well as in studies with patients' biomarkers, suggesting a potential linking mechanism between amyloid β accumulation and tau pathology (33).

In experimental studies, increased hyperphosphorylation of tau protein has been associated with higher degree of tau accumulation and greater AD severity (19). The accumulation of neurofibrillary tangles may be responsible for impaired axonal transport, synaptic attenuation, mitochondrial and cytoskeletal dysfunction, and oxidative stress (31).

Pathological changes

AD pathology is characterized by deposition of amyloid β in plaques and accumulation of neurofibrillary tangles; the former are extracellular aggregates of Aβ42 and Aβ40 fibrils, while the latter consist of intracellular aggregates of hyperphosphorylated tau protein (34).

The accumulation of neurofibrillary tangles begins in the allocortex of the medial temporal lobe (entorhinal cortex and hippocampus) and spreads throughout the isocortical regions, mainly in the associative areas with a relative initial sparing of the primary motor, sensory and visual areas (35).

Unlike the neurofibrillary tangles, amyloid plaques accumulate mainly in the isocortex, while hippocampus, entorhinal cortex, basal ganglia, brainstem and cerebellum are less affected by amyloid pathology (35).

Neurofibrillary degeneration has been shown to be related more strongly to the severity of cognitive impairment than amyloid pathology (35, 36). Nonetheless, this finding is contingent on the form of amyloid being evaluated (20). While a study from the AD Neuroimaging Initiative did not find any associations between amyloid accumulation detected by the amyloid PET and clinical progression (37), soluble Aβ oligomers in cerebrospinal fluid (CSF) (38) and in post-mortem brains (39,40) have been related to AD onset and progression of the cognitive impairment.

In addition to amyloid plaques and neurofibrillary tangles, other pathological features such as neuropil threads, dystrophic neurites, astrogliosis, synapsis damage, neuron loss, mitochondrial hypometabolism, oxidative stress, dysfunction of axonal transport and microglial activation have been reported in AD (35,41). These pathological alterations determine neurodegeneration, which can be observed in the structural neuroimaging as regional and global atrophy (42).

AD has typically been associated with a damage of the brain's gray matter; however, in the last few decades, neuropathological and neuroimaging studies have focused on the micro- and macro-structural changes of the white matter, suggesting that not only the neuronal damage, but also white matter degeneration and demyelination may be additional characteristics of AD pathology (42).

Loss of myelin has been reported in AD, and the brain regions most vulnerable were the areas that myelinate later (43,44), such as telencephalon, entorhinal cortex, hippocampus and the amygdala (45,46). Compared to non-demented controls, brain tissues of AD patients showed lower amounts of myelin basic protein, myelin proteolipid protein, 2',3'-cyclic nucleotide 3'-phosphodiesterase and cholesterol, and increased total fatty acid content (47).

These alterations of the white matter might be the result of damage to oligodendrocytes (48), which are vulnerable to Aβ accumulation (47,49), iron deposition (48,50), oxidative stress (50,51) and hypoperfusion (50). Moreover, also age-related DNA damage in myelinating oligodendrocytes may result in myelin loss (52).

Iron accumulation represents a relevant pathological alteration of the brains affected by AD, as it has been seen to be co-localized with amyloid plaques in post-mortem and neuroimaging studies (53). A recent neuroimaging study of our own group reported higher iron concentrations, detected by R2* relaxometry, in the basal ganglia and in the cortex of a large cohort of AD patients, when compared to age-matched controls; moreover, in AD patients, increased R2* relaxation rates in the temporal lobe were able to predict cognitive decline, independent of atrophy (54). It has been hypothesized that iron accumulation in cortical and subcortical regions might increase the levels of amyloid β, hyperphosphorylated tau protein and reactive oxygen species, contributing to neurodegeneration (53).

In addition to these pathological changes, also neuroinflammation might play an important role in AD. Chronic inflammation in the brain is caused by activated microglia cells, which are responsible for the release of several cytokines and toxic products, including reactive oxygen species and nitric oxide (55).

Microglia are the main immune cells of the central nervous system, characterized by a ramified configuration with small somas; they are inactive in physiological condition, while they become active in presence of threats to the central nervous system, such as disease or injury (55).

It has been hypothesized that the accumulation of amyloid β might be the main driver of activation of microglia (55). Receptors located on the surface of microglia, such as triggering receptor expressed on myeloid cells 2 (TREM2) or toll-like receptors might recognize amyloid β and induce its phagocytosis (56). However, after a prolonged time, the activated microglia cannot remove efficaciously the amyloid β, and continue to release pro-inflammatory cytokines (55).

This increased pro-inflammatory stage may contribute to tau hyperphosphorylation, by activating several kinases, and to accumulation of amyloid β (56), leading to neurodegeneration and exacerbation of AD pathology (55).

Clinical features

AD is characterized by a progressive impairment of memory and cognitive function as well as development of neuropsychiatric symptoms.

A decline in the cognitive functions emerges already in the symptomatic pre-dementia phase, which is defined as mild cognitive impairment (MCI); however, unlike AD, cognitive deficits in MCI do not substantially interfere with daily activities (57). It has been observed that the prevalence of MCI in the population older than 65 years old is about 10-20 %, it increases with age, and it is higher in the male sex and in presence of risk factors (57).

Memory impairment, mainly affecting the episodic memory, is considered the leading symptom of AD. In addition to memory loss, AD patients present progressive deficits in at least one other cognitive domain, resulting in aphasia, apraxia, agnosia or impairment of the executive functions (58).

It is noteworthy to distinguish early onset AD, which includes about 5% of all AD cases and is frequently caused by genetic mutations, from late onset AD, which typically occurs in subjects older than 65 years old (59). Early onset AD has usually a more aggressive disease course and is characterized by better memory function but worse executive function as well as more impaired attention and visuospatial skills, in comparison to late onset AD (59).

Almost all AD patients develop neuropsychiatric symptoms such as apathy, depression, delusions, hallucinations, aggression, and sleep disturbances (60). Neuropsychiatric symptoms may occur already in the stage of MCI and may be related to a faster progression to AD (61). Depression and apathy are the most frequent symptoms in patients with MCI and early AD, while verbal and physical agitation as well as delusions and hallucinations may increase with the progression of the disease (61). Neuropsychiatric symptoms have been associated with reduced quality of life and increased hospitalization as well as caregiver burden (61).

Patients with AD have an increased prevalence of epileptic seizures, compared to normal elderly population (62). Seizures may be present in all the AD stages, but they are more frequentin the late phases of the disease (62). Diagnosis of epilepsy in AD may be difficult since non-convulsive seizures, which are the most frequent in AD, might be masked by the impaired cognitive function of the patients (62). Myoclonic seizures have been observed to be more common in genetic forms of AD, particularly in the advanced phases of the disease (62).

In the last period of AD, patients are completely dependent from their caregivers. Language is reduced to single words and even early biographical memories can be lost (63).

Because of apraxia, basic motor functions such as swallowing and chewing may be impaired, resulting in dysphagia (63). It has been observed that patients with AD have also sensory deficits caused by dysfunction of the temporoparietal lobes, resulting in delayed oral transit ofthe liquid substances, decreased taste and smell sensation, and impaired swallowing function (64). Dysphagia can lead to dehydration, malnutrition, loss of weight and appetite, cachexia, functional decline, pneumonia *ab ingestis*, higher hospitalization rate, increased caregiver burden and shorter survival time (65).

Malnutrition is very frequent in patients with AD due to the impairment of swallowing and to the reduced taste sensation and loss of appetite. It has been reported that in AD patients reduced nutritional status related significantly to increased behavioral psychiatric symptoms of dementia, independently of cognitive impairment (66).

In AD, death occurs on average 8.5 years from the clinical onset of the disease (67); pneumonia *ab ingestis*, myocardial infarction and septicaemia are the most common causes of death in AD (63).

Biomarkers

In AD, the pathological alterations in the brain begin decades before the onset of symptoms, as seen in studies with patients with dominantly inherited AD (68). For this reason, the identification of reliable biomarkers may have an important role in the early diagnosis of AD in clinical routine and research (69). Moreover, biomarkers may play a significant role in the development of new pharmacologic treatments as additional diagnostic measures in subjects clinically considered as having AD and as indirect measures of disease severity in longitudinal studies, in order to investigate the effect of the studied drug on its targets (70).

In the last few years, several biochemical, neuroimaging, neuropsychological and genetic markers for AD have been investigated; however, only a limited number of CSF and neuroimaging biomarkers are widely used and have been included in the new diagnostic criteria (71). The majority of the investigated biomarkers have focused on the core pathological characteristics of AD, while others explored non-AD related features, such as neurodegeneration or inflammation (72).

While CSF biomarkers assess the quantity of proteins in the CSF at a given point of time, neuroimaging measures evaluate the neuropathological damage accumulated over time (71) and its regional distribution (73).

According to the current AD criteria, biomarkers are divided into three groups based on respective AD pathological alterations that they measure (71). Amyloid-related changes are detected by low CSF concentration of Aβ-42, reduced Aβ-42/ Aβ-40 ratio as well as by abnormalities at the amyloid-PET; biomarkers of fibrillary tau are increased CSF phosphorylated tau (P-tau) and positive tau PET scan, and neurodegeneration is diagnosed by elevated total tau CSF concentrations, atrophy on MRI and hypometabolism on the [(18)F]-fluoro-deoxyglucose positron emission tomography (FDG-PET) (71).

The development of these CSF and neuroimaging biomarkers, which currently play a very important role in the identification of AD-related neuropathological changes, are example of advancements the biomarkers detection and validation (74). Nonetheless, their use as effective first-line methods for the identification of AD pathology may be limited by their invasiveness, their lack of accessibility and their high cost (74). Therefore, research is now focusing on the identification of blood-based biomarkers, which may represent potential cost-effective methods for large-scale screening for AD in primary care settings (74).

CSF biomarkers

Three CSF biomarkers have been shown to have high diagnostic accuracy for AD: the 42-aminoacid form of Aβ (Aβ42), the total tau (t-tau) and the phosphorylated tau (P-tau) (75).

AD is characterized by low CSF concentrations of Aβ42, due to cortical amyloid deposition (75). The notion that the amount of Aβ42 in the CSF is valid marker of brain Aβ-plaque load is supported by several studies (76). Low concentrations of CSF Aβ42 have been associated with clinical diagnosis of AD and amyloid pathology in post-mortem brains (77,78), and with amyloid imaging findings in PET studies (79–82). By dividing the CSF concentration of aggregation-prone Aβ42 by soluble β40, diagnostic accuracy can be improved (83). The ratio Aβ42/ Aβ40 has been observed to be superior to the use of the single concentration of CSF Aβ42 in the identification of AD patients (84).

The detection of lower CSF concentrations of Aβ42 in patients with negative amyloid PET scans suggests that CSF Aβ42 identifies cortical deposition of amyloid before amyloid PET and, therefore, may be very important for individuals with suspected early AD-related pathology (84) .

In opposition to Aβ42, CSF concentrations of t-tau and P-tau proteins are elevated in AD (75). While CSF t-tau seems to reflect the progressive accumulation of damaged tau due to the neuronal loss in AD brains (85), higher CSF concentrations of P-tau have been related to formation of neurofibrillary tangles in the neocortex (86).

Increased CSF levels of tau and P-tau proteins have been associated with faster disease's course and reduced survival time in severe AD patients (87). Higher CSF concentrations of total tau and P-tau have been further associated with worse cognitive function, interesting particularly memory and orientation skills, and with a more rapid cognitive decline in AD patients (88).

Further, it has been reported that CSF tau and P-tau concentrations were related to brain atrophy, mainly in the medial temporal lobes (89). Similarly, a significant correlation was foundbetween increased CSF levels of t-tau as well as P-tau and higher tau tracer uptake, principallyin the temporal lobe and in the temporoparietal junction, at the PET scans of patients with AD(90).

The concentration of Aβ42, tau and p-tau in the cerebrospinal fluid can be quantified by techniques such as enzyme-linked immunosorbent assay (ELISA) (91).

A recent meta-analysis showed that the use of these three CSF biomarkers was accurate to discriminate not only AD patients from healthy controls, but also subjects with mild cognitive impairment due to AD from patients with stable mild cognitive impairment (75).

Similarly, Hansson et al. reported that the combination of CSF concentration of total tau protein and Aβ42 had a sensitivity of 95% and a specificity of 83% for early identification of AD pathology in patients with MCI (92).

In addition to these biomarkers, other CSF markers for AD are currently studies, but further research is need to understand if they are reliable and accurate for AD diagnosis.

The concentration of neurogranin, a dendritic protein which mediates the synaptic long-potentiation, has been seen to be elevated in AD and to have a strong correlation with severity of cognitive impairment, grade of atrophy and reduced glucose metabolism at the PET scans in the early stages of the disease (91). It has been observed that neurogranin is an accurate biomarker for neurodegeneration and synaptic loss (91).

Along neurogranin, neurofilaments are candidate biomarkers for the inclusion in the "N" category of the A/T/N classification (93). Neurofilaments, intermediate filaments contained in the central and peripheral nervous system, are composed by three subunits, depending on the molecular weight: the neurofilament (Nf) light chain, the Nf medium chain and the Nf heavy chain (93).

Neurofilaments are involved in the preservation of the cell shape and, in neurons, are important for the modulation of axonal diameter, contributing to the control of the neuronal response to external stimuli (93). Neurofilaments are released into the extracellular space after axonal damage, causing a rise in their CSF concentrations (93). For this reason, neurofilaments subunits, particularly the Nf light chains (NfL) which are more abundant and soluble in the CSF, have been suggested as marker of axonal damage (93).

Increased CSF levels of NfL were found in AD patients and in subjects with MCI in comparison with cognitively unimpaired individuals (94). A significant positive correlation was observed between CSF concentrations of NfL and CSF levels of tau and p-tau, but not of Aβ-42; moreover, higher CSF NfL levels related to reduced volumes of the cortical grey matter and of the hippocampus as well as to increased amyloid load at the PET scans and lower MMSE scores (94).

Similarly, Andersson et al. observed that higher CSF concentrations of NfL were associated with increased amyloid plaque load in the cortex in mice models and with greater uptake of ^{18}F-

flutemetamol at the amyloid PET scans of patients with AD (95). A recent meta-analysis observed that higher CSF concentration of NfL was accurate in discriminating patients with AD and normal controls (93).

Neuroimaging biomarkers

Brain imaging has an important role in the identification *in vivo* of structural and functional changes related to AD pathophysiology. Despite enormous research efforts over the past several years, only few neuroimaging biomarkers are currently available and widely used in clinical practice.

MRI is a non-invasive and easy accessible tool for the study of brain morphology, at a macro- and microscopic level. In current AD criteria, MRI is used to evaluate brain atrophy, which likely reflects the loss and shrinkage of neuropil and is considered a marker of neurodegeneration (71). The role of MRI in the study of AD pathology is further discussed in the next chapter.

FDG-PET measures glucose uptake of neurons and glial cells, and can reliably detect AD patterns of brain metabolic dysfunction, even in the early phases of the disease (96). Typically, FDG-PET scans in AD patients show hypometabolism in temporo-parietal regions, precuneus and posterior cingulate cortex (96). A good correlation between ante-mortem FDG-PET diagnosis of AD and post-mortem validation has been reported by Hoffman et al. (97).

Although FDG-PET can help in early differential diagnosis of neurodegenerative brain diseases (98), it only recognizes nonspecific alterations in parenchymal glucose uptake and not identifies the substrates underlying the AD pathology (99). In the last years, several radiotracers targeting specific aggregates for amyloid-β and tau protein have been developed (99).

Amyloid PET detects in vivo Aβ deposition with high sensitivity and specificity . Three fluorinated amyloid PET tracers (^{18}F-florbetapir, ^{18}F-florbetaben and ^{18}F-flutemetamol) have been approved for clinical use (100). In a meta-analysis, the sensitivity of amyloid PET for AD diagnosis has been found to range between 60% and 100%, with the majority of studies reporting sensitivities of 90% or greater (99).

Recently, PET imaging tracers have been developed also for the identification *in vivo* of tau pathology. Among the tau PET ligands, F-flortaucipir is the only approved by the U.S. Food

and Drug Administration (101) and has been shown to bind selectively to paired helical filament tau and TAR DNA-binding protein 43 (TDP-43) aggregates in post-mortem brain tissue (102). It has been observed that F-flortaucipir is able to distinguish accurately patients with AD from normal controls and other neurodegenerative diseases (103).

Blood biomarkers

Blood biomarkers may have an important role in clinical routine as well as in the clinical trials because of their greater accessibility and reduced costs (74). However, their development presents some difficulties because of the complexity of blood, which includes cellular components and several molecules contained in the extracellular fluid (74). Moreover, the blood-brain barrier limits the detection in blood of potential biomarkers of AD pathology, which may exist in blood at very low amounts (74) . Further, the concentration of the biomarkers in blood may be modified by liver degradation, renal excretion and by the co-existence of other diseases which may alter the plasmatic protein profile (74).

Reduced $A\beta\text{-}_{1\text{-}42}$ and $A\beta\text{-}_{1\text{-}40}$ plasma concentrations, measured by an ultrasensitive immunoassay (Simoa), have been reported in patients with AD dementia compared to subjectswith subjective cognitive impairment or MCI, and to cognitively unimpaired individuals (104).

In AD patients, weak but significant positive correlations were reported between blood and CSF concentrations of $A\beta\text{-}_{1\text{-}42}$ and $A\beta\text{-}_{1\text{-}40}$, while negative correlations were found between plasma levels of $A\beta\text{-}_{1\text{-}42}$ and cortical amyloid deposition at the PET scans (104).

Patients with prodromal or preclinical AD showed lower plasma levels of $A\beta\text{-}_{1\text{-}42}$ and of $A\beta\text{-}_{1\text{-}42}/\beta\text{-}_{1\text{-}40}$ ratio; nonetheless this reduction was less pronounced compared to the marked decrease seen in the CSF, suggesting that AD-related pathological changes may be detected in CSF years before AD pathology appears in blood (104).

Increased levels of tau protein in plasma have been found in patients with AD compared to cognitively unimpaired individuals or subjects with MCI, while no differences were reported between patients with MCI who converted to AD during the follow-up time and normal controls, suggesting that higher tau concentrations may be detected in the plasma in a later stage of the disease (105,106).

A weak correlation was found between plasma tau levels and CSF Aβ-$_{1-42}$ values, while no association was reported between plasma and CSF tau concentrations, suggesting that plasma tau may reflect only some aspects of AD-related pathology (105,106).

Higher levels of tau in plasma related in AD to cognitive impairment, increased atrophy rate and decreased hypometabolism at the FDG-PET, and in MCI to accelerate cognitive decline (105).

A promising blood-based biomarker for AD-related neurodegeneration is the neurofilament light, which is released both in the blood and in the cerebrospinal fluid and represents a measure of axonal injury. (107,108).

Higher plasma levels of NfL were observed in AD patients in comparison to controls and subjects with MCI, and in MCI patients compared with controls (107). Plasma amounts of NfL in patients with AD were associated with increased CSF levels of tau, p-tau and NfL, and with higher plasma concentration of tau and reduced CSF Aβ-$_{1-42}$ values (107).

Neuroimaging studies reported that higher plasma NfL levels were associated with greater atrophy of the grey (107,108) and white matter (108) , and with increased uptake of amyloid and tau tracer uptake at the PET scans (108). Further, it has been observed a positive correlation between plasma NfL and grade of cognitive decline (107).

Temporal ordering of biomarkers alterations

The temporal ordering of biomarkers abnormalities is crucial for better understanding AD pathology. A hypothetical model of the dynamic of AD-related biomarkers has been proposed by Jack et al.(76).

In this model, deposition of Aβ plaques begins early in the disease's course, reaching a plateau before neurodegeneration and clinical symptoms appear. The notion that Aβ-plaques deposition occurs before cognitive impairment is supported by several studies that found abnormalities of amyloid biomarkers in approximately 20-40% of cognitively normal elderly (76). These findings are in line with post-mortem studies which reported AD pathological changes in the brains of cognitively unimpaired individuals (109).

Longitudinal studies investigating healthy controls and patients with MCI and AD using repeated MRI and amyloid PET scans observed a constant slow rate of amyloid deposition across all the groups, in contrast to the rate of ventricular enlargement, which was significantly higher in AD patients than in the control group and in the subjects with MCI (37). Further, ventricular enlargement, but not amyloid deposition at the PET, correlated with cognitive decline (37). These findings further suggest that the accumulation of amyloid plaques occurs decades before the symptoms´ onset (76).

Biomarkers of neuronal damage and dysfunction, such high CSF concentrations of p-tau protein or positive tau-PET, become abnormal later in the disease and correlate with dementia severity (76).

It has been observed that neurofibrillary tangles may be found also in absence of symptoms; however, in cognitively intact individuals the presence of neurofibrillary tangles is usually limited to the entorhinal cortex, while in patients with AD is more widespread (76). Conversely, amyloid deposition as plaques may be diffuse throughout the brain also in asymptomatic people (76).

Brain atrophy on MRI is the last biomarker to appear abnormal and is strongly related to cognitive impairment in both MCI and AD (76). Higher atrophy rates have been reported in patients with AD compared with cognitively unimpaired individuals (76). MRI showed a greater association with cognitive and functional decline than the other biomarkers in the later stages of the disease (76).

Because of the different temporal dynamic and anatomical pathways of biomarkers' abnormalities, at a given time point, different brain regions will be at different phases of the disease (76). This advantages neuroimaging biomarkers over CSF biomarkers, since imaging can reveal the different stages of the disease both temporally and anatomically (76).

Diagnostic criteria

The first diagnostic criteria for AD were published in 1984 by a working group established by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's disease and Related Disorders Association (ADRDA). These criteria proposed to diagnose AD as definite, probable and possible, according to the medical history and the clinical features. Additionally, a diagnosis of definite AD required histopathological confirmation (110).

In the following years, the National Institute on Aging (NIA) together with the Alzheimer's Association (AA) proposed new criteria (NIA-AA) based on the distinction between AD pathological changes and the clinical expression of the disease (111). Histopathological studies, indeed, showed that AD plaques and neurofibrillary tangles were also present in the brain of subjects who were not cognitively impaired (112–114). In contrast with the previous criteria where a post-mortem examination was required, AD pathological changes could now be identified *in vivo* by measuring biomarkers (111).

The NIA-AA criteria were revised in 2018 and proposed a biomarker-based definition of AD in living people (71). Cognitive symptoms were not mandatory, defining AD exclusively by its underlying neuropathological changes that can be demonstrated *in vivo* by biomarkers, obtained through CSF analysis (Aβ 42 or Aβ 42/Aβ 40 ratio; total tau; phosphorylated tau), PET (Amyloid- or Tau-PET) or structural MRI (atrophy) (71).

Diagnosis of AD is performed with the "ATN" classification, which considers three groups of biomarkers according to the pathologic process that each measures ("A" refers to amyloid deposition, "T" to tau pathology and "N" to neurodegeneration) (71).

In base to these criteria, diagnosis of AD requires the alteration of both tau and amyloid biomarkers, while the presence of neurodegeneration is optional (71). In case of amyloid pathology in absence of abnormalities in the tau biomarkers, the label "AD pathologic change" would be used (71)

AD and "AD pathological change" are considered as earlier and later stages of "AD continuum", independently of cognitive status (71).

Therapy

Disease-modifying treatments, able to alter the underlying disease pathology or disease course, are not yet available (1). All currently approved therapies for AD (Acetyl-cholinesterase inhibitors and Memantine) are symptomatic therapies that aim to improve cognitive and neuropsychiatric symptoms without modifying the underlying course of the disease (115).

Acetyl-cholinesterase inhibitors (donepezil, galantamine and rivastigmine) inhibit the cholinesterase enzyme from breaking down acetylcholine, rising both the level and duration of the neurotransmitter action (116).

Memantine is a low affinity N-methyl-D-aspartate receptor antagonist and has been approved for moderate to severe AD (115). It works by decreasing L-glutamate excitatory neurotoxicity, which has been seen to be involved in the AD pathophysiology (117).

A recent meta-analysis has observed a modest but significant benefit of concomitant treatment with acetyl-cholinesterase inhibitors and memantine over the use of monotherapy with acetyl-cholinesterase inhibitors in patients with moderate to severe AD (118).

In addition to pharmacologic therapy, several non-pharmacologic treatments and behavioral strategies may be useful in order to improve neuropsychiatric symptoms and reduce caregiver burden (119).

The use of antipsychotics should be limited to those situations when non-pharmacologic interventions have failed (119).

Magnetic Resonance Imaging in Alzheimer's disease

Neuroimaging methods such as MRI play a crucial role in the study *in vivo* of the AD-related neuropathological mechanisms and in the clinical identification of AD (120). The research of reliable MRI biomarkers for AD is very important, since MRI, in comparison to PET, has greater accessibility, higher resolution and lower repeat measurement error (121).

While conventional MRI of the brain has an important role in discriminating AD from other pathologies, neuroimaging research is now focusing on the development of non-conventional MRI methods that may help to provide new insights into the earliest microstructural AD-related pathological abnormalities in vivo (122).

The following subchapters will give a brief description of the role of conventional and non-conventional MRI methods in the diagnosis of AD.

Conventional MRI

Conventional MRI is considered to be an important tool in diagnostic algorithms of dementias, since it can help in the identification of other causes of cognitive impairment, such a subdural hematoma, tumors, vascular malformation, normal pressure hydrocephalus and inflammatory, metabolic, toxic or infective processes (123).

Although conventional MRI methods can identify preclinical damage in regions particularly vulnerable to AD, such as hippocampus and entorhinal cortex (124), these findings lack specificity and should be considered in the context of the patient's age and clinical examination (123).

Distinguishing between the different forms of neurodegenerative dementias is very important to let the patients to access appropriate support and care (125); this will be even more relevant as soon as disease-modifying therapies will be available (126).

Conventional MRI sequences are important for the detection of brain atrophy and other static tissue abnormalities, such as white matter hyperintensities (WMH).

Measurements of brain atrophy

Brain atrophy is one of the main features of AD-related pathological changes and has been included in the new diagnostic criteria for AD (71). The deposition of amyloid plaques and neurofibrillary tangles in AD is considered to increase neural and synaptic loss, resulting in cortical atrophy (127).

High-resolution T1-weighted MRI sequences present high contrast between gray matter, white matter and cerebrospinal fluid, allowing the segmentation of cortex, deep gray matter structures and ventricles (128). For this reason, T1-weighted MRI sequences are optimal for studying brain shrinkage (129).

Several methods have been developed in order to evaluate brain atrophy in AD using conventional MRI.

Visual rating scales represents semi-quantitative methods for the atrophy assessment in regions particularly vulnerable to AD neuropathological changes, such as the medial temporal lobe; they are quick to apply and not require specialist software and expertise (130). Among the visual rating scales, robust reliability has been reported for the Scheltens' scale (130); this 0-to-4 points score focuses on the height of the hippocampus as well as on the width of the choroid fissure and of the temporal horn (131).

In the last few decades, several techniques for the quantitative assessment of the global and regional volume have been developed, ranging from manual methods to fully automated approaches.

Manual methods require expert investigators to segment a brain region based on anatomical conformation and, before any study using the manual segmentation is started, intra- and inter-rater analysis are required (132). In contrast, semi-automated and automated quantitative methods, do not need a manual tracing of brain regions, resulting in a higher time-efficiency (132).

Among the fully automated techniques, the voxel-based morphometry is the most used; it assesses differences in regional brain concentration through a voxel-wise comparison of multiple brain images (133). Primary advantages of voxel-wise methods are that they are independent from operator and do not necessitate any *a priori* hypothesis on the anatomical localization of brain atrophy (134).

Further, many software packages, such as FreeSurfer, have been developed for the quantitative image analysis. FreeSurfer is a freely available, open source image analysis tool that allows not only the volumetric segmentation of most macroscopically visible brain regions, but also the mapping of the thickness of cortical gray matter (135).

In post-mortem studies, brain atrophy, assessed by structural MRI, has been demonstrated to be a valid marker of neurodegeneration due to AD (136). A strong correlation has been found between brain atrophy on MRI and neurofibrillary degeneration (137,138). The deposition of hyperphosphorylated tau protein follows the Braak staging (46) and has been associated with the pattern of regional gray matter loss observed on structural MRIs of patients with AD (139).

Brain atrophy involves very early the medial temporal lobe structures such as entorhinal cortex and hippocampus (128), after affecting the cortex along a temporal-parietal-frontal trajectory, with relative sparing of the sensorimotor and primary visual cortices until the later stages of the disease. This topographical progression correlates with the chronological onset of symptoms and with the different stages of dementia severity (140,141). It has been shown that atrophy in the medial temporal lobe can reliably discriminate patients with AD from age-matched healthy controls with sensitivities and specificities greater than 85% (122).

Longitudinal studies showed that the rate of atrophy in the prodromal stage of AD is at least twice than in normal aging; this finding has been reported for the whole brain, but even more for the hippocampus and entorhinal cortex (142). In a large multicenter study, the rate of the whole-brain loss in AD was approximately three times higher than the rates in the healthy controls (1.5% vs 0.6% pro year) (143). Similarly, a meta-analysis showed that hippocampal atrophy rates were significantly greater in AD patients (mean rate: 4.66%) than in normal controls (mean rate: 1.41%) (144). In AD, higher rates of brain atrophy and ventricular expansion related to disease progression (143,145–148). Moreover, the rate of volume loss in several structures, including entorhinal cortex, hippocampus, and temporal lobe, related to cognitive decline in patients with AD (144).

The assessment of the amount and rate of atrophy may have an important role also on the identification of subjects with MCI as well as prediction of MCI to AD conversion. Recently, atrophy in specific brain regions, namely the “AD signature regions”, has been associated with incident dementia and mild cognitive impairment (MCI) as well as progression from MCI to dementia, independent of other dementia risk factors (149). These “AD signature regions” include the hippocampus, the parahippocampal cortex, the entorhinal cortex, the inferiorparietal lobule, the precuneus and the cuneus (149).

Further, higher rates of whole brain atrophy and ventricular enlargement over 1-2 years were related not only to greater risk of MCI and dementia in cognitively normal subjects but also to higher risk of conversion from MCI to dementia (150). In another study, baseline volumes of whole brain, ventricles, hippocampus and entorhinal cortex as well as their respective volume loss rate (enlargement rate for ventricles) were associated with earlier conversion from MCI to AD (142).

However, although structural MRI may contribute to AD diagnosis, brain atrophy measures show low specificity since it may include effects of aging and other neurodegenerative diseases and processes (151). Despite the fact that several studies reported volume loss in the whole brain, in the medial temporal lobe, in the hippocampus and in the entorhinal cortex of AD patients, structural MRI in a recently published systematic review showed low sensitivity and specificity and could not be recommended in clinical practice for early diagnosis of AD in patients with MCI (152).

An additional method for the assessment of grey matter atrophy in aging and in neurodegenerative diseases is the study of cortical thickness. This is supported by post-mortem studies that observed that cytoarchitectural changes, typical of aging and neurodegenerative processes, affect ultimately the thickness of the cortex (153–155). The analysis of cortical thickness using MRI represents the study *in vivo* of the changes in the width of the cortical gray matter layer that covers the surface of the brain (156,157).

Lower cortical thickness on structural MRI has been reported in patients with AD in comparison to MCI subjects and healthy controls (158). Querbes et al. found that the normalized thickness index, a measure of cortical thickness computed with a subset of zones optimal for the discrimination between stable and progressive MCI subjects, could discriminate patients with AD from healthy controls with an accuracy of 85%, and could correctly predict the evolution to AD for 76% of subjects with amnestic MCI (159). Cortical thinning has been further associated with brain amyloidosis (158,160,161) and cognitive impairment in AD (158).

Measurements of white matter lesions

Fluid attenuated inversion recovery (FLAIR) MRI images allow the visualization of abnormalities of cerebral white matter, such as WMH (128). According to visual rating scales, WMH are classified into periventricular or deep (162). Periventricular WMH are contiguous to the ventricles and have been associated with ependymal loss, differing degrees of myelination in adjacent fiber tracts and cerebral ischemia with associated demyelination (163). In contrast, deep WMH are located apart from the ventricles in the subcortical white matter and seem to be related to microcystic infarcts and patchy rarefaction of myelin which are ischemic in nature (164)

Several techniques may be used in order to measure the presence and severity of WMH on MRI; thy range from visual rating scales to semi-automated and fully automated techniques.

Among the visual rating methods, the Fazekas scale is the most used because of its simplicity and good reliability (165). This scale grades the deep and periventricular WMH on a 3-point score according their size and confluence (162).

In contrast to visual rating methods, semi-automated and automated techniques can provide exact WMH volumes and have more reliability. However, semi-automated quantification are labor intensive and time consuming, and automated methods are not optimal either since they need manual correction for improving accuracy (166).

WMH are frequently found in post-mortem histological examinations of brains from both demented and cognitively intact elderly (167). The burden of WMH has been associated with increasing age (168), with faster cognitive decline and with higher risk for dementia in the general population (169).

Higher WMH load has been found in patients with AD in the periventricular (162,170–172) as well as in the subcortical frontal and parietal white matter (172). Periventricular WMH load increased with the progression of AD (169) and related to higher risk of conversion from MCI to AD (166,173) as well as of incident AD in the general population (174,175).

Non-conventional MRI

AD-related pathology builds up for years before macroscopical changes can be seen through conventional imaging (176) and clinical symptoms appear (177). Recently, new MRI techniques have been developed for the study of tissue abnormalities at a microscopic level. The development of these new MRI methods aims to quantify *in vivo* the properties of tissue elements, such as myelin, axons, dendrites, glia, and to identify pathological features such as demyelination, inflammation, axonal loss (178). The detection of these early neurodegenerative alterations may be critical for improving diagnosis, staging and monitoring the response to the treatment (176).

The following subchapters aim to give a brief overview of the main non-conventional MRI techniques.

Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) is a MRI technique based on the noninvasive measurement *in vivo* of the diffusion of water molecules in neural tissues, providing information on the orientation and integrity of white matter tracts (179).

DTI considers two measures for the study of the changes to barriers to diffusion: the fractional anisotropy (FA), which detects the degree of directionality of water diffusion, and the mean diffusivity (MD), which represents the mean water diffusion rate (180,181). Recently, other DTI measures such as axial and radial diffusivity have been included in order to assess the rate of water diffusion along the longitudinal and perpendicular axis, respectively (180,181). While increased axial diffusivity has been related to axonal degeneration, higher values of radial diffusivity have been associated with demyelination (180,182).

In AD, DTI studies reported microstructural alterations not only in grey matter regions (183), but also in the white matter (184,185), particularly in the temporal and frontal lobes, in the corpus callosus, in the posterior cingulum, in the superior longitudinal fasciculus and in the uncinate fasciculus (186).

It has been observed that adding DTI measures of posterior cingulum to hippocampal volume increased consistently the accuracy in discriminating AD and MCI from healthy controls, from 63% to 74% in MCI, and from 78 to 91% in AD (183).

Longitudinal studies showed that the measurement of diffusivity in hippocampus was superior to the assessment of hippocampal volume in the prediction of conversion from MCI to AD (187,188). Further, several studies showed that alterations of DTI metrics were associated with cognitive impairment in AD (184,189–192).

Free water imaging

Free water imaging is a diffusion MRI post-processing technique that extracts free water from diffusion MRI obtained by DTI acquisitions, resulting in better assessment of tissue-specific parameters, such as FA, in areas of partial volume effect with CSF or edema, and enabling more comprehensive fiber tracking in healthy and pathological conditions (193). Abnormalitiesin the FA measures within the edema following removal of free water give reliable informationabout the underlying microstructural alterations (193).

Higher free-water values have been found in the left hippocampus of MCI subjects compared to healthy controls; moreover, free-water measures of both left and right hippocampi were related to cognitive function and to Aβ1-42 CSF levels, suggesting that this innovative MRI method may provide important information about neurodegenerative processes in AD (194).

Compared to DTI, free water imaging showed higher sensitivity to early microstructural alterations in white matter related to AD (195). Increased free-water values in the white matter have been associated with AD diagnosis (196) and poorer cognitive performance in AD and MCI (195,197).

Quantitative susceptibility mapping

Quantitative susceptibility mapping (QSM) is an recent MRI method that is sensitive to magnetic susceptibility variations between tissues (198). The data is acquired typically using flow-compensated gradient echo images, with a set of parameters that depend on the tissue in question (178). Tissue differences in the magnetic susceptibility of brain tissue might be related to variations in iron, calcium, lipids and myelin (199). Post-mortem MRI studies showed that magnetic susceptibility, assessed by QSM, was related mainly to iron content in the deep grey matter, while in the white matter regions QSM was not accurate for the assessment of iron concentrations (200).

Abnormal iron deposition in deep gray matter has been proposed as marker for tissue damage in several neurodegenerative diseases, including Parkinson' and Alzheimer' disease (200). Higher iron load was related to increased amyloid deposition and tau-related pathology in AD (201). Higher susceptibility in the deep grey matter was observed in AD patients (202,203) and has been associated with worse performance on the cognitive tests, and with increased amyloid deposition as well as off-target tau binding at the PET (204).

R2 relaxation rate*

R2* relaxation rate (=1/T2*) is an MRI technique which allows to measure tissue iron content in vivo. This method has been validated in post-mortem study, showing a strong correlation between R2* values and chemically determined brain iron concentrations in both grey and white matter (200).

Recently, a work of our own group explored with R2* protocol the amount of regional iron in a large cohort of AD patients compared with healthy controls. Patients with AD showed higher iron concentrations in the basal ganglia as well as in the global cortex, and regionally in the occipital and temporal lobes. In the AD cohort, the authors found an increased iron accumulation over time in basal ganglia and a significant association was reported between iron deposition in temporal lobe and cognitive decline over a mean follow-up time of seventeen months (54).

Magnetization Transfer Imaging

Magnetization Transfer Imaging is a method used to improve image contrast in MRI (205); it has been first applied by Wolff and Balaban (206) and is now used in research for the investigation of brain development, aging and disease (207,208).

Apart from angiography applications, MTI has been scarcely used in clinical routine, due to the longer acquisition process, compared to standard T1- and T2-weighted imaging (207).

This technique will be fully reviewed in the next chapter, as it is the focus of our research project.

Magnetization Transfer Imaging

The following subchapters will give an overview on MTI and on its applications for the study of Alzheimer's disease.

Basic principles of MTI

For MTI we here consider two populations of hydrogen protons: the hydrogen protons in the free water and the protons bound to the macromolecules, such as proteins and lipids (209). The signal measured with MRI comes from the free protons, which have sufficiently long T2 relaxation times so that spatial encoding gradients can occur between excitation and acquisition before the signal has been completely decayed. On the contrary, the protons bound to the macromolecules are too decaying too fast to be detected directly by conventional MRI sequences (208). Nevertheless, thanks to their constant motion, the free protons frequently exchange magnetization with macromolecular proteins, influencing then each other's state (134). The bound proton fraction can thus be observed indirectly by utilizing the dipolar coupling and chemical exchange mechanisms that cause magnetization transfer between both proton populations (129).

In MTI, an off-resonance radio frequency pulse induces the saturation of the magnetization of the bound protons, in order to reduce their magnetization to zero (210). The restricted motion of protons results in very short T2 relaxation rates and leads to a much broader absorption line shape of the bound protons in comparison to the free protons (208).

For this reason, the macromolecular protons can be preferentially saturated by an appropriately placed radio frequency pulse (210). The effects of this selective saturation are partially transferred to the liquid protons via magnetization exchange, leading to lower signal intensity on the magnetic resonance image (210). This resulting signal reduction in regions where free and bound protons are exchanging energy is at the basis of magnetization transfer contrast. The amount of signal attenuation is assessed by the calculation of magnetization transfer ratio (MTR) (186).

MTR measures the magnetization exchange related to the extent of the bound protons fraction, and is calculated through the formula:

$$\mathrm{MTR}=\frac{M0-MSS}{M0}$$

where Mss and Mo are the signal intensities obtained with and without magnetization transfer saturation, respectively (211). MTR is the most used MTI parameter because it is easy and fast to calculate. Moreover, since MTR is a quantitative measure, it is reproducible and comparable among subjects (212).

Several methods for the analysis of the MTR images have been described (213).

A common approach to assess global MTR changes is histogram analysis, which takes both microscopic and macroscopic lesions of the examined tissue into consideration (213). An MTR histogram is a map of the frequency of each MTR measure over a sufficiently large span of MTR values that are observed in the whole brain or in a given tissue segment (212). Histogram analysis consider several parameters, such as average MTR as well as the height and position of histogram peak, which represents the most common MTR value (212).
In the case of whole brain analysis, the height and the position of the peak are determined mainly by the white matter, since white matter has higher MTRs than grey matter (212). Microstructural changes would reduce the height but not the position of the peak as long as most white matter regions remain intact (212). Cortical atrophy and partial volume effects can also influence the peak height, since MTR of the CSF is very low (212). The MTR histogram is a highly automated method and has low intra-rater, inter-rater and scan-rescan variability (129).
MTR changes in regions such as WMH or segmented brain tissue areas can be explored using a region of interest (ROI) analysis (214). In contrast to the latter, histograms analysis has the advantage to be operator-independent and to summarize the information from a whole image into few simple numerical indices (214).

Another method useful for the assessment of the tissue damage of the white matter lesions is the analysis of the mean MTR, which is based on the calculation of the average MTR of the T2- or T1- visible lesions (213).

Further, the voxel-based approach of the analysis of MTR images allows an overall assessment of macroscopic and microscopic alterations of the whole brain or specific brain regions, preserving spatial information of lesion location, in contrast to histogram analysis (213). In order to identify gradients and edges of abnormal MTRs that are too subtle to be detected bythe conventional MTR maps, an approach based on the contour plotting of MTR can be used (213); this delineates abnormal MTR regions based on statistical variation from normal (215).

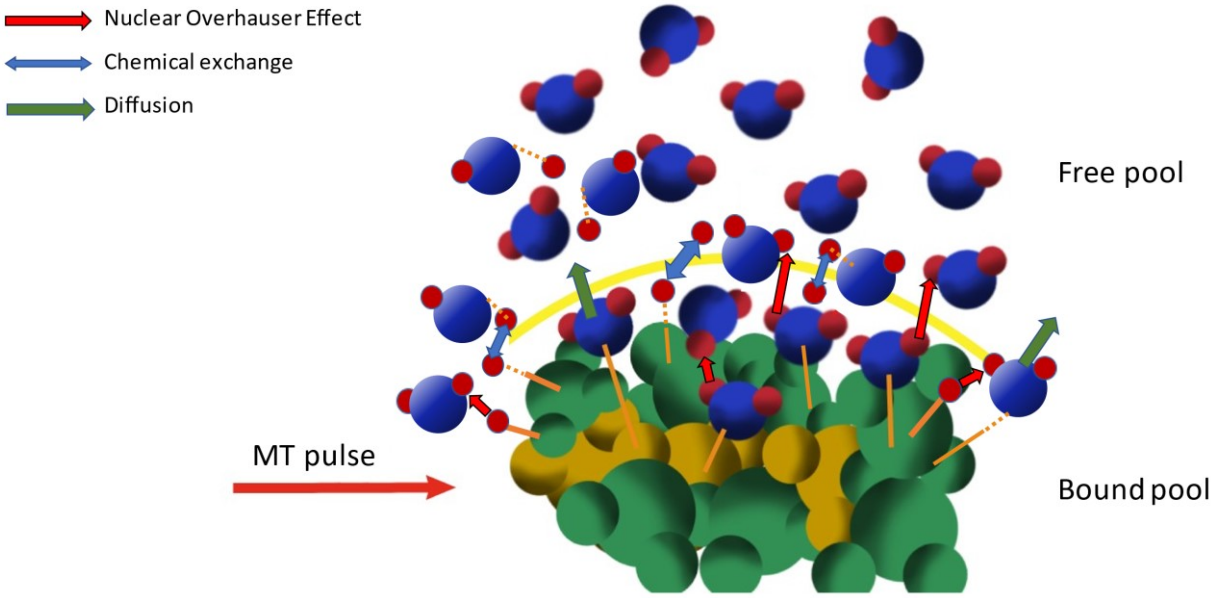

Figure 1. Physical principles of magnetization transfer

The transfer of energy from the "bound pool", which includes molecules of water bound to the macromolecules such as myelin, to the "free water" after the application of a radiofrequency magnetic field, called magnetization transfer pulse, is at the base of the magnetization transfer mechanism. The fundamental principles of magnetization transfer are: **nuclear Overhauser effect** (red arrows), which is the exchange of magnetization between a proton bound to the macromolecules and an adjacent water molecule; **chemical exchange** (blue arrows), which consists in the dissolution of the protons bound to the macromolecules into the free pool; and **diffusion** (green arrows), which is represented by the diffusion of the water molecules adjacent to the macromolecules into the free pool (own figure).

MTI and histopathological correlates

Post-mortem studies on multiple sclerosis (MS) brains have been conducted in order to identifythe associations between MTR measures and histopathological changes.

Schmierer et al. (216) studied the relationship between measures of myelin content, axonal density as well as of gliosis, and quantitative MR metrics in postmortem tissues of twenty individuals with MS. A strong correlation was found between MTRs and myelin content, whereas only a moderate association was seen between higher MTRs and greater axonal count.The significance of this relationship disappeared when myelin content was included in the regression model; the correlations between myelin content and MTRs, and between demyelination and axonal loss, could explain the association between MTR and axonal count. Further, MTRs were significantly increased in remyelinated than in demyelinated lesions (216).

Waesberghe et al. (217) investigated the histopathological changes of MS lesions, detected by MRI, in a post-mortem sample of seventeen MS patients. In this study, MTR values were significantly lower in macroscopically normal tissue with reactive changes than in lesions withno activity; moreover, a strong correlation was found between MTR metrics and the degree ofaxonal density.

In a work with post mortem spinal cord of MS patients, Mottershead et al. (218) observed that higher MTRs related to greater axonal density and myelin content. Although MTR loss is considered mainly as a marker for demyelination, the authors reported that MTR metrics showed a stronger correlation with axonal count than myelin density.

MTI findings in Alzheimer´s disease

Magnetization transfer abnormalities were seen in the MRI scans of patients with AD. Ridha et al. found MTR reductions in histogram analysis in the whole brain (WB) and in the hippocampus, but not in the pons or in the parietal white matter of AD patients (219). Both WB volume and WB mean MTRs were independently related to AD diagnosis; the assessment of both parameters raised the sensitivity of differentiating AD patients from healthycontrols to 83% from 50% for WB volume alone and 33% for WB mean MTR alone (220). However, it is noteworthy to mention the small size of this study (n=18) and the not significantdifference in diagnostic value between hippocampal MTRs and hippocampal volume. Lower MTR measures in the WB histograms of AD patients have been also reported in other works (221,222).

Kabani et al. (222) found lower mean MTR values within the whole gray and white matter of patients with AD. Using histogram analysis, Bozzali et al. reported lower MTR peak heights in the global cortical grey matter in 18 AD patients compared to 16 healthy controls (223).

Van Es et al. (224) found decreased histogram peak heights in the grey as well as in the white matter of AD patients, while mean MTR values were lower only in the grey matter. Focusing on the regional analysis, the same research group found that AD patients had reduced grey and white matter peak heights in all lobes than the subjects with normal cognition; similarly, mean MTR values of all regions, except the occipital white matter, differed between the two groups (225).

Reduced MTR peak heights in the temporal and frontal lobes were reported in AD also by Van de Flier et al. (221).

A study using voxel-wise analysis (226) found decreased MTR values in the hippocampus and in the amygdala bilaterally (prominent in the left side, but also significant in the right side) in patients with mild AD, but not in MCI subjects, when compared with healthy controls.

In another study, hippocampal mean MTR values could discriminate healthy controls from patients with very mild AD (Clinical Dementia Rate=0.5) with a sensitivity of 75% and a specificity of 90%, showing an overall discrimination rate of 85% (227). In the corpus callosum of AD patients, reduced MTR values were found in the anterior and posterior portions, while regional atrophy of the corpus callosum was seen only in the posterior part (228,229).

So far, only one longitudinal study used MTI in order to follow the progression of the neuropathological changes in AD over time (230). Twenty-eight patients with mild to moderate AD and nineteen healthy controls underwent clinical examination and MRI scans at 1.5 Tesla over a follow-up time of 12 months. At the baseline, reduced global MTR peak height and hippocampal mean MTR were reported in AD patients when compared with controls. No significant differences were found in thalamus, putamen and caudate nucleus. MTR declined significantly globally and in all the considered regions over the follow-up time and was paralleled by a brain tissue loss of 2.2% per year (220).

Only few works explored the value of MTR in discriminate AD from other types of dementia (227,231). Hanyu et al. (227) reported that hippocampal MTR values were significantly reduced in AD in comparison with patients with non-AD dementia and healthy controls, and no statistically significant differences in hippocampal MTRs were found between patients with non-AD dementia and subjects with normal cognition. MTR measures (discrimination rate= 77%) were better than visual rating (discrimination rate = 65%) in discriminating AD from other types of dementia.

The same group observed, in another work, lower MTRs in the hippocampus, parahippocampus, and posterior cingulate white matter of individuals with AD and Lewy Bodies dementia, when compared with age-matched subjects with normal cognition (231). Further, hippocampal MTRs could discriminate patients with AD from individuals with Lewy Bodies dementia, showing a sensitivity of 76% and a specificity of 71%. These findings could be explained by different underlying histopathological substrates, with less severe neuronal degeneration in the hippocampus of patients with Lewy Bodies dementia (231).

MTI in Mild Cognitive Impairment

The detection of microstructural changes, which occur years before the loss of tissue volume, may permit an earlier identification of AD pathology. An earlier AD diagnosis may be very important in order to identify appropriate groups of "at risk" subjects for clinical trials and for the use of disease-modifying therapies, when they will be available (232). So far, only few studies used MTI for the identification of microstructural alterations in MCI.

While no statistically significant differences were found in the volumetric measures between MCI subjects and healthy controls, lower mean MTR values were reported in the global grey matter, but not in the white matter, of MCI patients (222).

In another study, peak height but not mean MTR values were reduced in the whole brain, in the grey matter and in the white matter of MCI subjects; in the same work, no MTR differences were seen between MCI and AD patients (224).

In the regional analysis, MCI subjects showed, in comparison to controls, lower white matter MTR peak heights in all the lobes, whereas the peak heights of grey matter MTR were reduced only in the frontal and temporal lobes. However, mean MTR of all the considered regions did not differ between individuals with MCI and controls, and between AD and MCI patients (225). Similarly, in another work, subjects with MCI showed lower peak heights of the temporal and frontal lobes than controls (221).

Using an automatic model-based magnetization transfer imaging, Wiest et al. demonstrated that microstructural changes detected in the entorhinal cortex could differentiate patients with AD and MCI with a sensitivity of 1.00 and a specificity of 0.94 (233). In another study, quantitative MTI of the hippocampal head, but not MTR values, could help to distinguish AD from MCI patients (234).

Association between MTI measures and cognition in AD

While several studies have explored the association between MTR measures and cognition in normal aging (129), so far only few works focused on the role of MTR in predicting cognitive impairment in patients with AD. Results can been seen in Tab 1.

The majority of the studies focused on the association between global MTR measures and cognitive deterioration, assessed by Mini Mental State Examination (MMSE); only two studies (221,235) included in their analysis more detailed neuropsychological tests, such as Cambridge Cognitive Examination (CAMCOG) (221) and accurate tests for memory and language function (235).

The results of these studies are conflicting. While only one study did not find any correlation between MTR values and cognitive impairment (219), the majority of previous works found a significant association between impaired global cognition and lower MTR measures in the whole brain (221), in the global grey matter (224)(230) in the hippocampus (230,236) in the temporal lobe (225), in the thalamus (230) and in the putamen (230). When considering the deep gray matter structures, the relationship between MTRs and MMSE was stronger for the left than for the right side (230).

Using more detailed neuropsychological investigations, Fornari et al. (235) found an association between regional MTR changes in the superficial WM and worse performance on memory and naming test. In contrast, the study by van der Flier et al. (221) did not show any specific relation between regional MTRs and different cognitive domains.

So far, only one longitudinal study explored the association between decrease of MTR measures and cognitive decline. Over an observational period of one year, no significant correlations were found between cognitive decline and changes of MTR histograms in the whole brain as well as in the hippocampus, putamen and caudate nucleus (230).

Table 1. **Studies on MTR measures and associations with cognitive impairment in patients with Alzheimer's disease**

Author	Cohort	Method	Brain regions	Findings
Ridha et al. (219)	18 AD 18 HC	MTR histograms Mean MTR	WB, hippocampus, parietal WM and pons	No significant correlations between MTR parameters and MMSE
van Es et al. (224)	55 AD 19 MCI 43 HC	MTR histograms Mean MTR	GM, WM	Across groups, significant associations between MMSE and peak heights in GM ($r = 0.53$, $P < 0.001$) as well as in WM ($r = 0.41$, $P < 0.001$), and mean MTR of the GM ($r = 0.37$, $P < 0.001$), but MMSE did not correlate with mean MTR of WM.
van Es et al. (225)	55 AD 19 MCI 43 HC	MTR histograms Mean MTR	Frontal, occipital, temporal and parietal lobes	Across groups, peak heights and mean MTR of the temporal GM were associated with MMSE (peak height: $\beta = 0.75$, $P < 0.001$; mean MTR: $\beta = 0.74$, $P < 0.001$)
van der Flier (221)	22 AD 13 MCI 28 HC	MTR histograms	WB, temporal and frontal lobes	Across groups, lower peak height in all the considered regions correlated with poorer performance on all the neuropsychological tests*, except for CAMCOG praxis and TMT-A.
Hanyu et al. (236)	35 AD 27 non-AD 23 HC	Mean MTR	Hippocampus	Significant associations between hippocampal mean MTR and MMSE ($r = 0.70$, $P < 0.001$) in the AD group.
Ropele et al. (230)	28 AD 19 HC	MTR histograms	Hippocampus, thalamus, putamen and caudate nucleus	Left hippocampus ($r = 0.57$; $P < 0.001$), right hippocampus ($r = 0.38$; $P = 0.048$), left thalamus ($r = 0.52$; $P < 0.001$), right thalamus ($r = 0.42$; $P = 0.029$), left putamen ($r = 0.7$; $P < 0.001$), right putamen ($r = 0.60$; $P < 0.001$) MTR significantly related to MMSE in AD.
Fornari et al.(235)	15 AD 15 HC	Mean MTR	Superficial WM (U-fibers)	Lower MTRs in the superficial WM related to poorer performance on MMSE and on tests for language function and memory

AD: Alzheimer's disease; HC: healthy controls; MCI: mild cognitive impairment; MTR: magnetization transfer ratio; WB: whole brain, GM: grey matter, WM: white matter; MMSE: Minimental State Examination; CAMCOG: Cambridge Cognitive Examination

*Neuropsychological tests used: Minimental State Examination, Cambridge Cognitive Examination (subtests for memory, praxis, orientation, language and gnosis), Wechsler Memory Scale, Alzheimer's Disease Assessment Scale, Letter fluency, category fluency (animals, jobs), Trail Making Test (part A and B), Digit Symbol test

Aims and scope

Alzheimer´s disease represents the most common cause of dementia. Thus, it has been identified as a major public health concern and as a research priority (13). Typically, the symptoms at the beginning of the disease are mild memory difficulties, which evolve then towards cognitive impairment and dysfunctions in the daily activities (237). Neuropathological brain changes have been seen to occur many years before the beginning of the clinical diagnosis (5). The existence of AD pathology biomarkers in a preclinical stage has been reported through the study of pre-symptomatic individuals presenting autosomal dominant mutation, whose AD biomarkers have been measured in the CSF or in brain with amyloid PET (238).

The research in this field is now focusing on the identification of reliable biomarkers for an early detection of AD, and for patients´ stratification and follow-up in clinical trials.
Although CSF biomarkers are commonly used in clinical settings, they require invasive procedures (134). Only few neuroimaging biomarkers have been approved for clinical use and most of them are still object of research (239).

The role of non-conventional MRI techniques, such as DTI and MTI, which allow the assessment of microstructural brain changes for the detection of AD–related tissue changes, is still unclear (220). Several DTI studies showed loss of white matter integrity in AD and related them to Tau deposition in specific regions related to AD (240). Only few studies used MTI to investigated microstructural tissue changes in AD (220).

In this research project, we evaluated the microstructural changes in a large cohort of participants with AD, by calculating MTRs in the global and regional grey matter, as well as in the normal appearing white matter and in the WMH. We compared global and regional MTRs between patients with AD and age-matched controls, and we further investigated the association between MTRs and cognitive impairment in AD. Finally, we evaluated, in the AD cohort, the longitudinal MTR changes and their relationship with cognitive decline after a mean observational time of 1.06 years.

Material and methods

Study participants

This prospective study included seventy-seven participants with AD from the longitudinal cohort study Prospective Dementia Registry Austria (PRODEM) (241). PRODEM, financed by the Austrian Alzheimer society, is a national dementia registry including non-institutionalized dementia cases, which collects clinical information, MR imaging, biobanking data with six months follow-up over a period of two years. The participants of our study originate exclusively from the Graz center since it was the only center where an MTI sequence had been incorporated in the MR imaging protocol (220).

Seventy-seven age-matched healthy control participants without neuropsychiatric disease were randomly selected from the Austrian Stroke Prevention Study, a large prospective longitudinal study of the healthy older adult population of the city of Graz, Austria (242).

All the individuals included in the study received a comprehensive clinical evaluation and 3T MR imaging on the same scanner with the same acquisition parameters (220).

The majority of the patients with AD who were included at baseline in our study (47 of 77) underwent a subsequent clinical, neuropsychological and neuroimaging assessment with the same MRI protocol as that one of the baseline and with a mean follow-up time and standard deviation of 1.06 ± 0.24 years.

Diagnostic criteria of AD dementia

Dementia was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) (243) and National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (110).

DSM-IV criteria, published in 1994, defined dementia as a combination of memory impairment and at least one of the following cognitive deficits: apraxia, aphasia, agnosia or impairment of executive functioning; this cognitive disturbance, which does not occur exclusively during the

course of a delirium, should affect significantly the daily activities and should show a progressive worsening compared to the prior level of functioning (243).

NINCDS-ADRDA criteria (110), published in 1984, aimed to establish a guide for the diagnosis of AD for research protocols; because of the insufficient knowledge about the disease, these criteria were not fully operational and had to be used with the other existing dementia criteria, such as DSM. AD was classified into probable, possible and definite. Probable AD showed a typical insidious onset of dementia with progression, in absence of any other systemic or brain diseases that could result in cognitive decline (110). Conversely, possible AD could be diagnosed in presence of other brain or systemic disease, but, on clinical judgement, AD should be considered the more likely cause of progressive dementia. A diagnosis of definite AD required histopathological confirmation (110).

The severity of dementia was determined according to the National Institute for Health and Care Excellence (NICE) guidelines (244). MMSE score defined the severity of cognitive impairment as follows: mild AD: MMSE 21–26, moderate AD: MMSE 10–20, severe AD: MMSE less than 10.

Neuropsychological assessment

The cognitive function of the participants with AD was assessed with the MMSE (245) and the Consortium to Establish a Registry for Alzheimer Disease (CERAD) test battery (246).

The MMSE is a brief diagnostic tool, which consists in 19 individual tests of 11 domains covering orientation, registration, attention or calculation, recall, naming, repetition, comprehension, writing, and praxis (247).

The CERAD battery, which has a longer administration time than the MMSE, includes the following subtests: Verbal Fluency, Modified Boston Naming Test, Word List Memory and Constructional Praxis. These tests assessed the main cognitive domains affected by AD, including memory, language, visuo-constructional ability, and general cognitive functioning (246).

Image acquisition

MR imaging was acquired on a 3T whole-body MR system (Tim Trio, Siemens) with a 12-channel head coil (220).

The MR imaging protocol covered a T2 FLAIR sequence (TR/TE/TI=10,000/69/2500 ms, number of slices = 40, section thickness=3 mm, in-plane resolution=0.86mm x 0.86 mm), a T2-weighted sequence with two echoes (TE1/TE2/TR = 10/72/5260 ms, number of slices = 40, section thickness = 3 mm, in- plane resolution = 0.86mm x 0.86mm), and a 3D T1-weighted magnetization-prepared rapid acquisition of gradient echo sequence with whole-brain coverage (TR/TE/TI = 1900/2.19/ 900 ms, flip angle = 9°, isotropic resolution = 1mm) (220).

In addition, MTI was obtained using a spoiled 3D gradient-echo sequence (TR/TE = 40/7.38ms, flip angle = 15°, number of slices = 40, section thickness = 3mm, in-plane resolution = 0.86mm x 0.86mm), which was performed twice, with and without a Gaussian-shaped radiofrequency saturation pre-pulse (220).

Image processing

MTR maps were obtained using the formula MTR = (Mo-Mss)/Mo, where Mss and Mo represent the signal intensities measured with and without MT saturation, respectively.

The cortical structures were segmented entirely automated using FreeSurfer for the calculation of regional MTR values, as previously described (248,249). The estimated total intracranial volume was used to normalize the volumes of all segmental structures (220).

An in-house built bash-script based tool, which offers a graphic summary of the segmentations overlaid over the T1-weighted picture, was used to perform a visual quality check for each scan.

Out of all the segmentations offered by FreeSurfer, we considered in our analysis six regions of interest, namely the “AD-signature regions” (149), which had previously been identified as vulnerable to early pathological changes related to AD, (220). These regions were the cuneus, hippocampus, precuneus, parahippocampal cortex, precuneus, entorhinal cortex, and inferior parietal lobule (149).

Regional masks were superimposed on the MT-weighted images, after affine registration with the T1-weighted scan with FSL FLIRT (220).

We performed an optimization of the mask segmentation utilizing a semiquantitative T2 map (250) computed from the mono-exponential decay of both echoes of the T2-weighted sequence and registered to the T1 sequence in order to detect and eliminate CSF-contaminated voxels in the registered cortical masks (220).

After a histogram analysis of the T2 image for each regional mask, voxel intensities beyond the whole width at half maximum of the histogram peak were designated as CSF-contaminated voxels and were thus removed from our analysis (220). Finally, median MTR measures were retrieved from the CSF-corrected brain regions with FSLSTATS (220).

Hyperintense T2 lesions on the FLAIR maps were segmented using an in-house-developed semiautomatic region-growing tool, in order to evaluate binary masks of WM hyperintensities (251). We further obtained the normal-appearing white matter (NAWM) mask by removing the manually segmented WMH masks from the total white matter mask calculated by FreeSurfer (220). The extent of WMH was evaluated using the Fazekas scale (162).

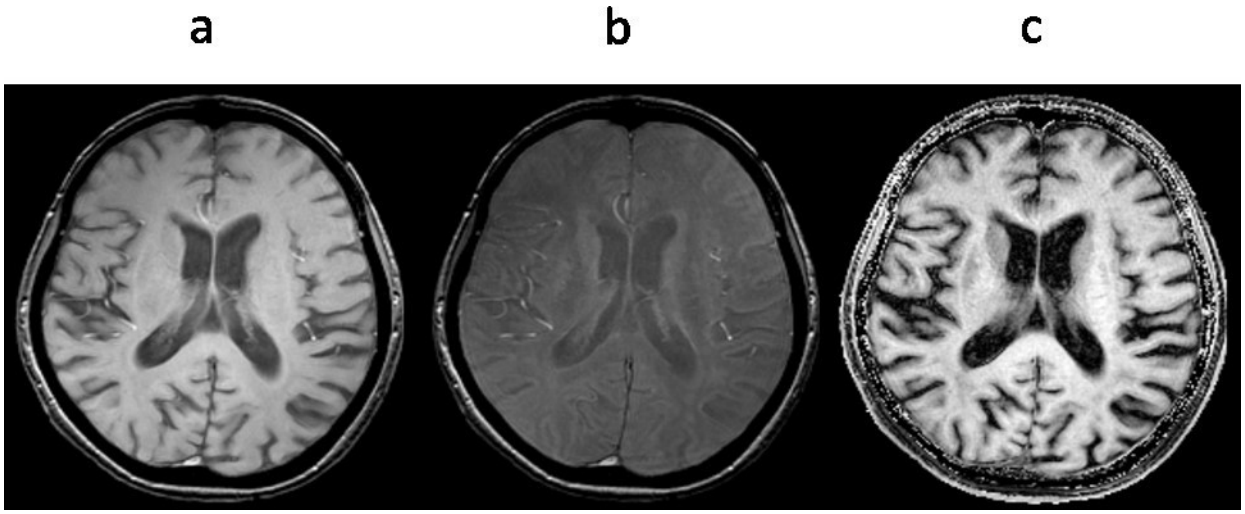

Figure 2. Example of calculation of MTR map in a 71 years old AD patient

The MTR map (c) can be determined from a reference scan (a) and from a scan obtained with the application of a magnetization transfer saturation (b). The signal intensity in the MTR map reflects the amount of magnetization transfer. Thus, brain areas with higher MTRs like white matter regions appear brighter than the cortex, which has lower MTR values (212) (own figures).

Statistical analysis

We used SPSS to analyze the data (version 25; IBM). The Kolmogorov– Smirnov test was used to examine the assumptions of normal distribution. To compare the two diagnostic groups we employed a paired t test in case of normal distribution, and a Wilcoxon signed-rank test for non-normally distributed samples.

Z-scores were computed for raw scores of MTR and normalized volumes, and they were used in the regression analyses (220). We used the one-sample Wilcoxon signed-rank test to explore whether the median annualized percentage rate differed from zero, as previously described (54).

The association between AD diagnosis and MTR measures in the global cortex, the AD-signature areas, the NAWM, and the WMH was investigated using logistic regression analyses (236).

Linear regressions with cognition as the dependent variable and MTR as the predictor variable were used to explore the correlations between MTRs and performance on the MMSE and CERAD subtests (220).

Similarly, linear regression models were performed for the study of the association between annualized MTR changes and cognitive decline over the observational time (220).

We considered age, sex, years of schooling, normalized regional volumes, and Fazekas score as potential confounders into all models (236).

The 95% percent confidence interval and the P value were calculated for each regression coefficient were calculated. A P value <0.05 was considered statistically significant (220).

Data Availability Statement

Anonymized datasets generated and/or analyzed during this study are available from the author I.C. upon reasonable request

Ethical Approval

The study was authorized by the Medical University of Graz's ethical committee (approval number: 19-135 ex 07/08) , and all participants or their caregivers completed informed permission forms.

Results

Study participants characteristics

A group of 156 patients with dementia were verified to be eligible and were included in the longitudinal cohort study PRODEM in Graz.

We ruled out from our study 19 participants since they presented other causes of dementia and 60 subjects whose MR examinations had no high enough MTI quality (Figure 2).

Seventy-seven patients with AD were considered in our study. They were 30 men and 47 women with a mean age of 72 ± 8 years, ranging from 51 to 87 years.

Fifty-five patients were affected by mild AD (MMSE score: range 21–28, mean ± SD: 23.98 ± 2.13), and 22 study participants were diagnosed with moderate to severe AD (MMSE score: range 14–20, mean ± SD: 17.14 ± 1.86) (220).

Further, we included in our study seventy-seven healthy age-matched controls (± 1 year). Their mean age was also 72 (±8) years, ranging from 51 to 87 years. MMSE score were superior to 24 for all control participants, with the exception of three subjects who scored 23 on the MMSE. However, none of the control participants presented symptoms of dementia or mild cognitive impairment (220).

The comparison of demographics, vascular risk factors, neuropsychological findings, and MRI measures between AD patients and healthy control individuals at the baseline are shown in Table 2.

No differences in the distribution of sex was observed in the two diagnostic groups ($P = 0.62$). Patients with AD scored significantly worse at the Minimental State Examination ($P < 0.001$), showed lower volumes of global cortex ($P < 0.001$) and of normal appearing white matter ($P = 0.01$), and had higher WM hyperintensity volumes ($P = 0.02$).

The 63.7% of the individuals with AD and the 40.3% of the cognitively intact subjects presented higher severity of chronic small-vessel disease, assessed by Fazekas grade II or III (220).

The majority of the patients with AD who were included at baseline in our study (47 of 77) underwent a subsequent clinical, neuropsychological and neuroimaging assessment with the

same MRI protocol as that one of the baseline after a mean follow-up time and standard deviation of 1.06 years ± 0.24. Their mean age was 71 years (standard deviation: 8, range 55-85), 55 % were female.

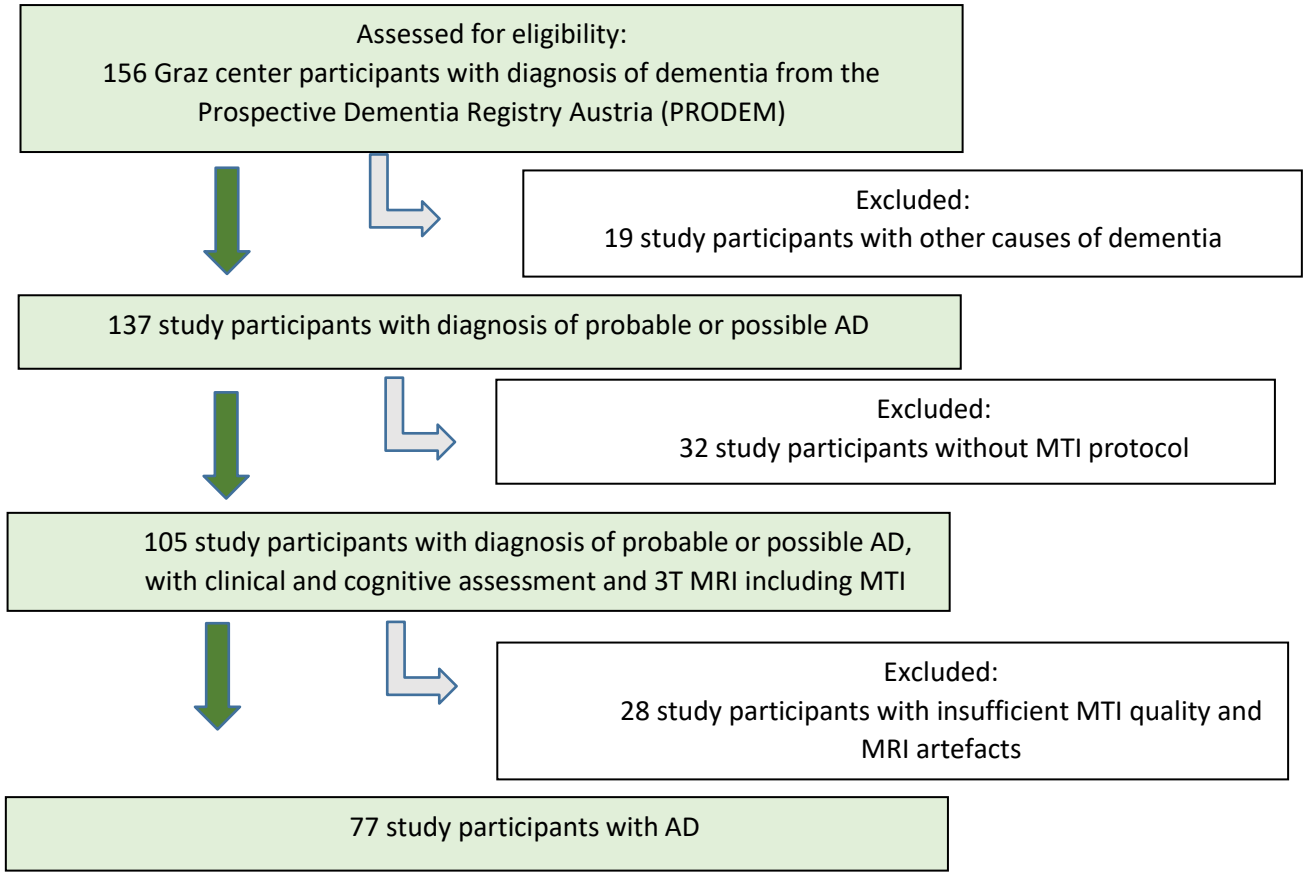

Figure 3. Flowchart shows the recruitment of the study participants with AD

Note. — AD: Alzheimer´s disease, MRI: magnetic resonance imaging, MTI: magnetization transfer imaging. Published in (220).

Table 2. Demographics, neuropsychological and MRI findings of study participants at the baseline

Study participants characteristics	AD (n=77)	HC (n=77)	P value†
BASIC DEMOGRAPHICS			
No. Female (%)	47 (61)	44 (57)	0.62
Age, years,*	72 (8)	72 (8)	0.98
Age Range, years	51-87	51-87	
Years of education*	11.12 (2.9)	11.25 (3.03)	0.56
VASCULAR RISK FACTORS			
Arterial hypertension N (%)	45 (58.4)	44 (57.1)	0.87
Diabetes N (%)	13 (16.9)	11 (14.3)	0.66
Heart disease N (%)	18 (23.4)	17 (22.1)	0.85
Hypercholesterolemia N (%)	40 (51.9)	39 (50.6)	0.87
NEUROPSYCHOLOGICAL TESTING			
MMSE*	22.03 (3.72)	27.57 (1.75)	**<0.001**
MRI VARIABLES			
Global cortex volume, cm3*	329.63 (14.53)	394.04 (41.25)	**<0.001**
Global NAWM volume, cm^3*	305.77 (63.71)	334.70 (52.52)	**0.01**
AD-signature regions volume, cm^3*	54.81 (10.15)	70.28 (8.86)	**<0.001**
WM hyperintensities volume, cm3*	16.19 (17.69)	11.64 (18.01)	**0.02**
Fazekas grade 2 or 3, no. (%)	49 (63.7)	31 (40.3)	**0.004**
Global cortex MTRs*	0.295 (0.016)	0.309 (0.008)	**<0.001**
Global NAWM MTRs*	0.384 (0.009)	0.388 (0.009)	**0.003**
WM hyperintensities MTRs*	0.322 (0.028)	0.350 (0.016)	**<0.001**
AD-signature regions MTRs*	0.297 (0.018)	0.309 (0.009)	**<0.001**

Note. — AD: Alzheimer´s disease, HC: healthy controls, MMSE: Mini Mental State Examination, WMH: white matter hyperintensities, MTR: magnetization transfer ratio

*Data are mean ± standard deviations

†Pearson $\chi 2$ for nominal, Wilcoxon and paired T-Test test for continuous variables were applied

Published in (220)

MTR in patients with AD and healthy controls

The study participants with AD showed lower global and regional MTR values in the cortex, in the NAWM, in the AD-signature regions and in the WMH than age-matched controls (Table 2, Figures 3 - 4).

WMH presented lower MTR values than NAWM, showing a MTR reduction relative to NAWM of 9.9% and 15.9% in the healthy controls and in the AD patients, respectively. The difference in MTR between NAWM and WMH was statistically significant larger ($P < 0.001$) in AD patients (mean MTR = 0.06) than in controls (mean MTR = 0.03).

No significant MTR changes in WMH and in grey matter structures were found with increasing WMH severity. In the AD cohort only, NAWM showed statistically significant lower MTRs ($P = 0.001$) in patients with higher WMH severity (Fazekas score 2 or 3) than in those with no visible white matter damage (Fazekas score 0 or 1), while no NAWM volume differences were seen between the two groups (Figure 5).

Lower MTR metrics in the global cortex, in the AD-signature regions, in the NAWM and in the WMH was significantly associated with AD diagnosis in the logic regression analysis, evenafter correction for age, sex, and years of education as well as regional normalized volume andextent of white matter damage (Table 3). In this logistic regression model, age, normalized regional volume and Fazekas score were overall significant independent predictors of AD diagnosis.

When analyzing the AD-signature regions singularly, we observed that reduced MTR values in the parahippocampal cortex, in the cuneus, in the precuneus and in the entorhinal cortex were related to AD (Table 4).

Moreover, lower MTRs in NAWM and WMH were related to AD independent of the severity of the dementia; conversely, in only the individuals affected by mild AD, reduced MTR values in the AD-signature regions (OR = 0.38, 95% CI:0.17, 0.82; $P = 0.02$) were associated with AD diagnosis.

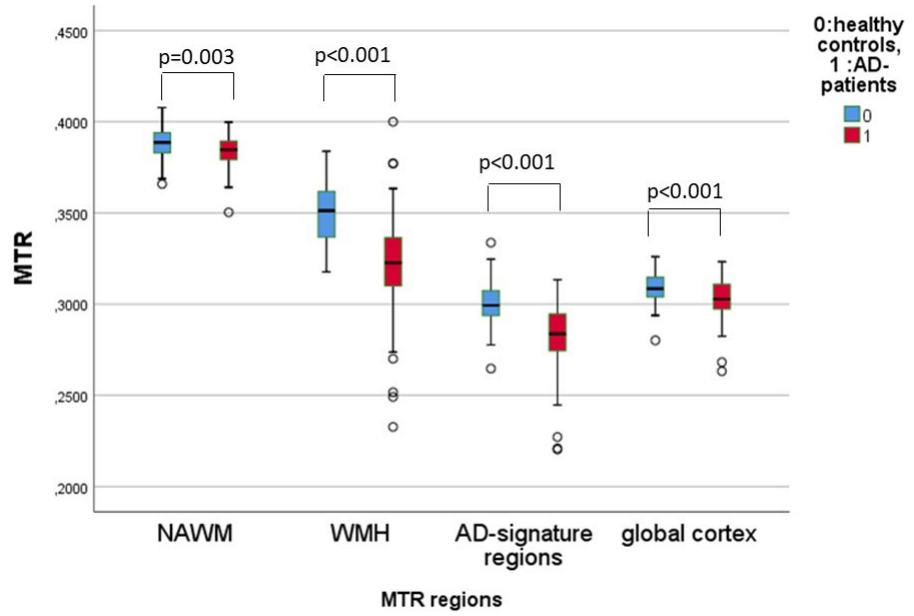

Figure 4. Clustered boxplot showing MTR values in AD patients and healthy controls

MTR values were statistically significant lower in AD patients than in healthy controls in all the considered regions.

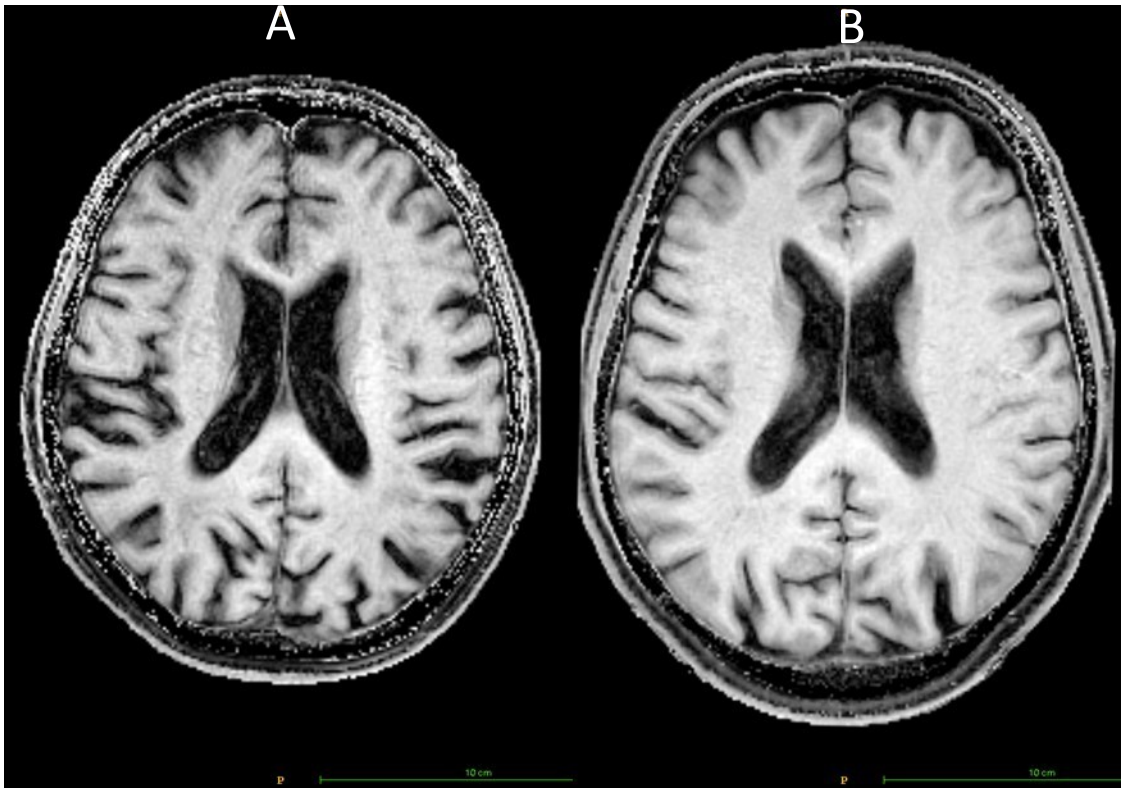

Figure 5. Example of MTR maps of a 71 years old patient with AD (A) and an age-matched healthy control (B)

The MTR map of the healthy control (B) shows higher signal intensity than the MTR map of the age-matched AD patient (A), where lower amount of magnetization (i.e. decreased MTR values) was observed (own figures).

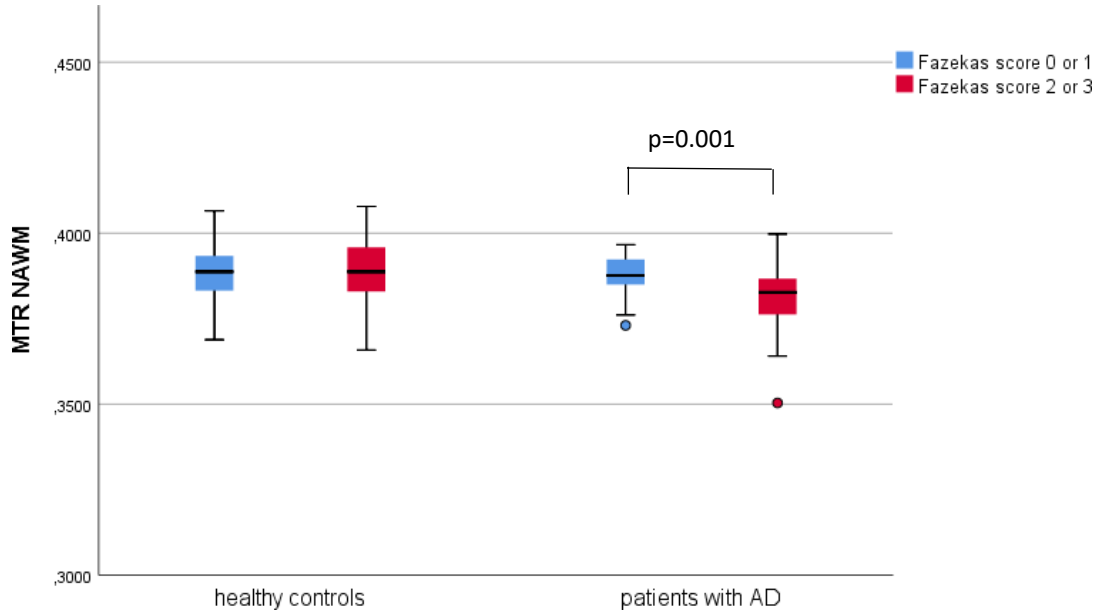

Figure 6. Clustered boxplot showing MTR values in the NAWM of AD patients and healthy controls in relation to WMH severity

AD patients with higher WMH severity (Fazekas score 2 or 3) showed lower MTR in NAWM in comparison to the AD subjects with no visible white matter damage (Fazekas score 0 or 1) (P = 0.001). No significant association was seen between MTR in the NAWM and WMH severity in the group of healthy controls (P = 0.79)

Table 3. Logistic regression analysis: lower MTRs relate to AD, independent of atrophy and white matter damage

MTR Regions	OR	95 % CI	P value†
Global cortex	0.47	0.22 ; 0.97	**0.04**
Global NAWM	0.59	0.39 ; 0.88	**0.01**
WMH	0.18	0.09 ; 0.33	**<0.001**
AD-signature regions	0.31	0.14 ; 0.67	**0.003**

Note. — MTR: magnetization transfer ratio, OR: odds ratio, CI: confidence interval, NAWM: normal appearing white matter, WMH: white matter hyperintensities, AD: Alzheimer´s disease

†corrected for age, sex, years of education, normalized regional volume and Fazekas score

Published in (220).

Table 4. Logistic regression analysis: lower MTRs in the AD signature regions relate to AD, independent of atrophy and white matter damage

MTR regions	OR	95 % CI	P value†
Hippocampus	0.71	0.43 ; 1.18	0.19
Cuneus	0.26	0.09 ; 0.73	**0.01**
Entorhinal cortex	0.57	0.35 ; 0.95	**0.03**
Inferior parietal lobule	0.45	0.19 ; 1.05	**0.06**
Parahippocampus	0.61	0.37 ; 0.99	**0.04**
Precuneus	0.24	0.12 ; 0.45	**<0.001**

Note. — MTR: magnetization transfer ratio, OR: odds ratio, CI: confidence interval

†corrected for age, sex, years of education, normalized regional volume and Fazekas score

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MTRs and cognitive performance

When considering AD patients and healthy controls simultaneously, we found that poorer global cognition assessed by MMSE (mean ± SD, range = 24.8 ± 4, 14-30) was associated with lower MTR values in the NAWM (β = 0.20; 95% CI = 0.18, 1.47; P = 0.013) and in the WMH (β = 0.35; 95% CI = 0.80, 1.98; P < 0.001), independent of demographic data, normalized regional volume and Fazekas score. In all the regression models, years of education and volume measures were independent predictors for MMSE.

However, such associations were not confirmed in the separate analysis for the two diagnostic groups. In the cohort of normal elderly, only age was significant predictor for global cognition, while in the AD group lower education level, but not age, was overall significantly associated with poorer performance on MMSE. Further, only grey matter MTRs related significantly to cognitive measures in AD; no such relationship existed for white matter MTRs.

Table 5 and 6 show the associations between MTRs and cognition in the AD group.

After correction for age, sex, years of schooling, normalized regional volume, and Fazekas score, the reduction in MTR measures in the global cortex was associated with poorer performance on the language subtest of the CERAD test battery.

Only a non-significant trend was seen for the association between cortical MTRs and MMSE (Table 5), and between MTRs in the AD signature regions and poorer performance on CERAD subtest for constructional praxis (β = 0.29; 95% CI = -0.01, 1.32; P= 0.05).

However, when analyzing the AD-signature regions singularly, lower MTR metrics in the cuneus and in the hippocampus were independent predictors for poorer global cognition, and reduced MTRs in the parahippocampal cortex, in the cuneus and in the entorhinal cortex were associated with worse constructional praxis (Table 6).

We did not find any significant associations with other CERAD subtests (data not shown).

No effect of laterality was observed on the MTR correlations with poor language function (data not show) (220).

Table 5. Linear regression analysis in AD: cortical MTRs relate to poorer language function

MTR regions	MMSE			Language function (Boston Naming Test)		
	β	**95% CI**	**P value †**	**β**	**95% CI**	**P value †**
Global cortex	0.23	-0.07 - 1.96	0.06	0.31	0.19 - 1.95	**0.02**
AD signature regions	0.14	-0.50 - 1.52	0.32	0.28	-0.02 - 1.74	0.05
Global NAWM	0.17	-0.24 - 1.67	0.14	0.11	-0.05 - 1.23	0.40
WMH	0.01	-0.78 - 0.88	0.91	-0.01	-0.81 - 0.67	0.89

Note. — MTR: magnetization transfer ratio; β: standardized regression coefficient; CI: confidence interval, AD: Alzheimer´s disease, NAWM: normal appearing white matter, WMH: white matter hyperintensities

†corrected for age, sex, years of education, normalized regional volume and Fazekas score

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Table 6. Linear regression analysis in AD: Lower MTRs in the AD signature regions relate to global cognitive impairment and poorer constructional praxis

MTR regions	MMSE			Constructional praxis		
	β	**95% CI**	**P value †**	**β**	**95% CI**	**P value †**
Hippocampus	0.29	0.03- 2.22	**0.04**	0.11	-0.45 – 1.00	0.45
Parahippocampal cortex	0.14	-0.43 - 1.37	0.30	0.38	0.28 – 1.41	**0.006**
Cuneus	0.32	0.26 - 1.95	**0.01**	0.45	0.51 – 1.56	**<0.001**
Precuneus	0.03	-0.77 - 0.99	0.80	-0.03	-0.63 – 0.52	0.85
Enthorinal cortex	0.09	-0.54 - 1.21	0.45	0.35	0.25 – 1.28	**0.004**
Inferior parietal lobule	0.11	-0.45 - 1.16	0.42	0.24	-0.13 – 1.31	0.11

Note. — MTR: magnetization transfer ratio; β: standardized regression coefficient; CI: confidence interval, AD: Alzheimer´s disease

†corrected for age, sex, years of education, normalized regional volume and Fazekas score

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Longitudinal assessment of MRI and clinical findings in patients with AD

MTRs decreased over time in the whole cortex. No significant MTR reductions over the follow-up time were seen in the NAWM and in the WMH (Table 7). Among the AD-signature regions, only the inferior parietal lobule was seen to decrease significantly over the observed time (annualized rate = −1.72%; P = 0.01).

On the contrary, volumes decreased significantly not only in the cortex, but also in the NAWM and in the AD-signature regions. No significant longitudinal changes were seen for WMH volume during the observation time (Table 7).

Longitudinal changes were observed for the scores of MMSE (median annualized rate, interquartile range = 2.77, 0.86 to 4.36; P < 0.001), and of CERAD subtests for language function (median annualized rate, interquartile range = 0.92, 0.00 to 1.09; P = 0.01) and verbal fluency (median annualized rate, interquartile range = 1.41, -0.86 to 3.00; P = 0.02), while constructional praxis did not worsen significantly over the observational time.

No significant associations were found between the annualized rate of MTR measures and cognitive decline (data not shown).

Table 7. Annualized MTR and volume changes in 47 participants with Alzheimer´s disease

Regions	MTR		Volume	
	Median (% per year)	**p value***	**Median (%per year)**	**p value***
Global cortex	-3.86 (-6.70 to 0.07)	**<0.001**	-56.96 (-60.64 to -50.09)	**<0.001**
Global NAWM	-0.65 (-1.94 to 0.42)	0.14	-11.27 (-21.81 to 2.51)	**<0.001**
WMH	0.93 (-3.51 to 3.23)	0.57	-0.02 (-0.18 to 0.24)	0.66
AD-signature regions	-0.65 (-3.39 to 3.5)	0.40	-5.80 (-11.81 to -1.80)	**<0.001**

Note.— Data are median values, and data in parentheses are the interquartile range. The annualized MTR rate calculated as follows: ([(FU-BL)/BL]/FU time)*100, where BL=baseline, FU = follow-up, FU-time = follow-up time (years).

MTR: magnetization transfer ratio, NAWM: normal appearing white matter, WMH: white matter hyperintensities, AD: Alzheimer´s disease

*Computed by using Wilcoxon signed rank test.

Discussion

In this cohort study, we confirmed the presence of global and regional microstructural changes both in the grey and white matter of a large cohort of individuals with AD. Alterations in the microstructure of the grey matter regions were related to poorer cognitive functioning and declined significantly over time in AD.

We used MTI at 3 Tesla MRI in order to explore brain microstructural changes and their association with cognitive function in AD.
While signal intensities obtained by conventional MRI are mainly based on the different relaxation characteristics of hydrogen protons in water molecules, MTI is a MRI technique useful for the investigation of non-water components in tissues and for the study of brain microstructure (212). Although several potential transfer mechanisms of magnetization have been identified, it is still unclear which one plays the dominant role in the central nervous system (212).

The AD-related microstructural changes were explored not only in the grey matter regions, but also in the normal appearing white matter and in the white matter lesions.
Due to the topographical distribution of the amyloid plaques and neurofibrillary tangles, AD has been typically regarded as a disease of the grey matter (48).
However, over the past few decades, neuropathological and neuroimaging studies highlighted the presence of alterations in the micro- and macrostructure of the white matter of patients with AD, indicating that white matter abnormalities may have an important role in the pathogenesis of AD (48).

In this context, histopathological studies found chemical alterations in the white matter of AD patients, consisting in higher total fatty acid content and reduced concentrations of proteins, cholesterol, myelin basic protein, myelin proteolipid protein and 2`,3`- cyclin nucleotide 3`-phosphodiesterase, and leading to loss of myelin integrity (252).
A diffuse damage of myelin has been reported in the first stages of AD, even in absence of infarction, Wallerian degeneration or amyloid angiopathy (253).
Demyelination may lead to an increased requirement of neuronal energy in order to keep unaltered the neurotransmission, resulting in higher production of free radicals (253).

The latter may then induce the oxidation of tau protein, causing its dimerization and polymerization into filaments (254), which are the precursors of neurofibrillary tangles (253).

Further, the presence of genetic alteration, oxidative stress, iron accumulation as well as vascular pathological changes may affect the functionality as well as the quantity and the DNA stability of oligodendrocytes or their precursors, which play an important role in the production of myelin or neurotrophic factors and in the modulation of neurite growth and neuronal connectivity (48). Although white matter changes occur early in the AD continuum, contemporaneously with the other AD pathological features, it is still unclear if these alterations are independent of each other (48).

Magnetization transfer imaging mirrors the exchange of magnetization between the freely mobile protons and those linked with macromolecules (216); in the central nervous system, the macromolecular density is mainly determined by myelin components (212), such as cholesterol (255), sphingomyelin (256) or galactocerebroside (257).
In line with this, we found higher MTR values in the white matter than in the grey matter structures in both patients and controls groups,. This has been already reported in previous works (224,225,258), further suggesting that MTR has high sensitivity for myelin (259) and white matter changes.

The association between MTR measures and myelin content has been supported by several studies. In a quantitative MTI study with mice treated with cuprizone, a neurotoxicant causing relatively selective myelin loss followed by spontaneous remyelination, Turati et al.(260) reported a significant correlation between the size of the restricted-motion pool and histological staining for myelin (Black Gold II) as well as immunofluorescent markers of myelin basic protein.
Similarly, Janve et al.(261) reported a strong association between quantitative MTI parameters and histological measures of myelin content in animal models of CNS demyelination, occurred after the intracerebral injection of lipopolysaccharide. Another work showed that the ratio of the pool size of macromolecular and free water protons, assessed by quantitative MT, was related to myelin content but not to axon injury in the optic nerve of a mouse model three days after retinal ischemia (262).

Histopathological studies, however, showed that MTR values are the result of the combination of several factors in addition to myelin content, such as inflammation (263), axonal content (216,217), pH (257,264).

In a post-mortem study, Schmierer et al. (216) investigated the association between quantitative MTR measures and brain histopathological changes of twenty patients with MS. The authors found a strong association between MTR measures and axonal count, as well as myelin density. Further, Mottershead et al. reported a stronger correlation between lower MTR values and axonal count, in comparison to myelin content (218).
Similarly, a strong association was found between MTR values and the degree of axonal count in a post-mortem sample of seventeen individuals affected by multiple sclerosis (217).

In a study with rat models of MOG-induced experimental autoimmune encephalomyelitis, Serres et al. observed a significant correlation between MTR lowering and infiltration of inflammatory cells (263). The increased water content, typical of inflammatory processes, may result in lower quantity of magnetization transfer by diluting the number of protons per unit volume of tissue, thus decreasing the MTR (265).

In our work, we found a reduction in MTR within the WMH relative to NAWM in the order of 9.9% and 15.9 % for healthy controls and AD patients, respectively. This reduction was modest but statistically significant marked in AD patients than in controls. Similarly, previous works found among non-demented subjects decreased MTRs in the WMH than in NAWM and this reduction ranged from 8% to 14.4% (259,265,266).
Although WMH are generally considered to be mainly related to small vessel cerebrovascular disease (267,268), post-mortem MRI studies demonstrated that WMH in MRI have different histological correlates (269). While punctate WMH have been associated with mild perivascular tissue damage (270), confluent WMH related to severe incomplete ischemic damage with axonal and myelin loss, reactive astrogliosis and lipohyalinotic arterioles (271).

To our knowledge, ours is the first study exploring MTRs in the WMH of patients with AD. Although the decrease of MTR in the WMH compared with NAWM was significantly higher in AD patients than in healthy controls, it was not as pronounced as that observed previously in MS plaques (258,272).

Previous studies showed that edema in absence of myelin loss may result in modest MTR reductions (273). Dousset et al. (258) reported that, in animal models of experimental allergic encephalomyelitis, acute lesions were mainly inflammatory with no evidence of demyelination and showed a reduction in MTR of 5-8% when compared with that of NAWM (258). In contrast, MS plaques had much lower MTR values than NAWM (average decrease of 26%), indicating that demyelination results in a larger MTR drop (258).

Our findings suggest that microstructural changes in white matter lesions of AD patients is more severe than those seen in non-demented subjects, but not so much as in the demyelinating plaques. It can therefore be speculated that edema and inflammation with only minimal myelin loss might be the underlying pathological substrate of MTR reduction within the WMH in AD. This is consistent with previous work that reported an association between microstructural changes in the periventricular WMH detected by DTI and peripheral inflammatory markers in patients with AD, but not in elderly controls (274).

Several authors reported that tissue structural changes spread further than the visible area of WMH, indicating that the underlying pathology is a more diffuse process affecting also the NAWM, and that the WMH are probably only the "tip of the iceberg" (275,276). Therefore, we investigated if higher WMH severity was associated with greater microstructural changes in the NAWM.

We observed that NAWM had lower MTR values in AD patients with higher Fazekas score (2 or 3) than in AD patients with no visible white matter damage; such relationship was not observed among the control participants.

These findings further suggest that the pathological substrates underlying WMH in AD and in normal aging might be different. This is in line with previous works, which proposed WMH as core feature of AD (271). A study on individuals with autosomal dominant mutations for AD observed that WMH volume was elevated already six years before the estimated age of symptom onset and correlated with Aβ1-42 concentrations in CSF (271). Similarly, other studies found an association between the amount of WMH and cerebral amyloid deposition (277) as well as increased tau concentrations in CSF (278).

Further, a histopathological study demonstrated that parietal WMH were associated with demyelination and axonal loss in AD as result of degenerative axonal loss, due to Wallerian degeneration triggered by cortical AD pathology (167); on the contrary, WMH in healthy controls related to demyelination only, as consequence of ischemic damage (167). The same authors reported that demyelination and axonal loss in the WMH as well as in the NAWM were

associated with greater WMH severity in AD, whereas in non-demented controls only myelin loss related to higher WMH load (167).

In this work, we observed lower MTRs in cortical and subcortical structures, in NAWM, and in WMH of a large number of AD patients, compared to a group of age-matched controls. Similarly, AD patients showed lower volumes of the cortex, the AD signature regions as well as the NAWM, while WMH volume was significantly increased in AD.
The reduction of MTR measurements and regional volume in AD was more pronounced in the grey than in the white matter, consistent with AD being a disease which involves predominantly the grey matter areas (222).

Previous work reported a reduction in the MTR measures calculated in the whole brain (219,221,224), in the cortex (222,223,225), in the hippocampus (226,230,236), in the amygdala (226), in the corpus callosum (228,229) and in the white matter (224) of patients with AD. No significant MTR reduction was found in the pons and in the parietal white matter (219).
In the regional analysis, reduced MTRs were observed in the frontal (221,225), in the parietal (225), in the temporal (221,225) as well as in the occipital lobes (225).
Focusing on the diagnostic accuracy, Ridha et al. found that whole brain MTR, when considered in combination with whole brain volume, could distinguish AD patients from healthy controls with a sensitivity of 83% (145). Such diagnostic value was not observed for the hippocampus (145).
By contrast, Hanyu et al. showed that hippocampal MTRs could differentiate healthy controls from patients with very mild AD with modest sensitivity (75%) and high specificity (90%) (236). Further, hippocampal MTRs could discriminate patients with AD from individuals with Lewy Body dementia with a sensitivity of 76% and specificity of 71% (231).

These previous studies, however, did not consider regional volume and severity of white matter damage as potential confounder. In our study we observed reduces MTR values in both grey and white matter regions, independent of atrophy and white matter lesions.

We further explored weather MTR metrics were related to AD diagnosis independent of disease severity. In our sample, fifty-five patients had mild AD, while twenty-two individuals were diagnosed with moderate to severe AD.

We observed that reduced MTR values in the normal appearing white matter and in the white matter hyperintensities were associated with AD diagnosis in study participants with both mild and moderately severe AD. By contrast, only in patients with mild AD, MTR metrics in the AD-signature regions related to AD.
We can hypothesize that these discordant relationships between grey matter MTRs in mild versus moderately severe AD might be explained by the fact that microstructural changes in the grey matter occur early in the AD continuum (220). However, the small sample size of study participants with moderate to severe AD might be responsible for low statistical power, resulting in lack of significant correlation between MTR in the cortical and subcortical grey matter areas and AD diagnosis (220).

We investigated the relationship between MTR values and global cognition for AD patients as well as for healthy controls. We hypothesized that microstructural changes may affect cognitive skills, by determining a dysfunction of the neural circuits which are essential to the normal cognitive performance. We used MMSE for the evaluation of global cognition for both controls and patients groups, and CERAD test battery for a more detailed neuropsychological assessment of the cognitive profile of the AD cohort.

Across all subjects, significant positive correlations were observed between MMSE score and MTR values in the NAWM and WMH, suggesting that alterations of the white matter microstructure may impair the normal brain function, determining cognitive deficits. However, such relationship was not seen when considering the AD cohort and the group of control participants individually. This might be explained by the smaller MMSE range within the single group.

While no significant associations were found between MMSE and MTR values in the control group, lower MTRs in the cuneus as well as in the hippocampus were associated with poorer global cognition in AD. Only a non –significant trend was observed between cortical MTRs and MMSE, while reduced MTRs in the cortex related to lower scores on the Boston Naming Test, which is a CERAD subtest for language function.

We further observed a strong association between lower MTR metrics in the parahippocampal cortex, in the cuneus as well as in the entorhinal cortex and worse performance on the CERAD subtest for constructional praxis.

The correlation between MTRs in the cortex as well as in the AD-signature regions and global cognition, language function as well as constructional praxis was moderate, but independent of brain volume and white matter lesions.

So far, only a few studies have looked at the association between MTR measures and cognitive functioning in individuals with AD (129) and they have had conflicting results.
While Ridha et al. (219) could not find any relationship between MTR values and cognitive skills, Van der Flier et al. (221) observed an association between poorer cognitive performance and lower peak heights of the MTR histograms in the whole brain as well as in the frontal and temporal lobes; however, the authors failed to find any significant relationship between regional MTR lowering and domain-specific cognitive deficits.
Similarly, Van Es et al. (224) showed that lower MTR histogram peak heights of grey and white matter related to poorer global cognition assessed by MMSE; however, in the ROI analysis, only grey matter and not white matter MTRs were associated with cognitive impairment (225). Within the subcortical structures, lower MTR values in the hippocampus (227,230), putamen (230) and thalamus (230) related to poorer performance on the MMSE.

In our study, we demonstrated that the association between MTRs and cognitive function in AD exists mainly with grey matter MTR and that it is independent of regional volume and white matter damage. Like others (219,224,225,279) we did not find a relationship between white matter mean MTR values and cognitive performance in AD.
We assessed the cognitive function of the control group using MMSE only. This represents a limitation of our work, since MMSE is not a sensitive method for the detection of cognitive deficits in cognitively normal individuals. However, a previous work of our own group exploring the relationship between MTR metrics and cognition in 355 non-demented individuals of the Austrian Stroke Prevention Family Study, found that lower MTRs of the normal appearing white matter related to poorer memory and executive function (280). The significant association between MTR metrics and cognitive functioning in normal aging but not in patients with AD may suggest that higher level of myelination may improve the cognitive performance in physiological condition, while in presence of pathological alterations the impact of myelination on cognition is no longer evident (281).

We further investigated the longitudinal MTR changes over a mean follow-up time of 1.06 years in the AD cohort, since the study of the temporal dynamic of AD-related microstructural

changes might aid in better understanding the pathological pathways of the disease and help for the development of new treatments.

So far, only one previous work explored the longitudinal changes of MTR in a small cohort of patients with mild to moderate AD (230). The authors of the study reported a decline of global histogram MTR metrics as well as of regional MTR measures in the hippocampus and deep grey matter structures over a follow-up period of one year (230).

We here extended this work by analyzing the regional MTR changes in the cortex, in the AD-signature regions, in the NAWM and in the WMH of a larger cohort of AD patients, using 3 Tesla MRI. We calculated MTR by performing ROI analysis, which allow the study of individual lesions and of discrete areas of brain tissue (213). We observed a significant MTR decline over time in the global cortex, but no statistically significant longitudinal MTR changes were found in the AD-signature regions, in the NAWM and in the WMH. This discrepancy might be explained by the fact that microstructural changes in the subcortical regions and in the white matter occur early in the AD pathology continuum, while cortical alterations are more widespread in the symptomatic phase of the disease.

In line with this, a previous DTI study proposed a biphasic model of AD pathology, suggesting that AD-related tissue changes affect first the regions most vulnerable to AD, as identified by previous works (282), and, only in the symptomatic stage of the disease, involve more widely the cortex (195). Similarly, abnormalities in the microstructure of whole-brain white matter were found in preclinical AD in absence of structural markers of brain atrophy, suggesting that microstructural alterations of the white matter may occur very early in the AD continuum (283). Consistent with these previous studies, we can speculate that the presence of longitudinal changes in the cortex, but not in the AD-signature regions or in the white matter, might be explained by the advanced stage of our patients' cohort in the AD continuum.

We explored the relationship between longitudinal MTR changes and cognitive decline over the observational time, failing in finding any significant association. This might be attributable to the fact that the patients who were evaluated after the follow-up time were those with less pronounced cognitive decline. So far, only one previous work investigated the correlation between MTR changes in the deep grey matter regions and decline of the cognitive function over a period of one year in 28 patients with AD (230) .Similarly to our study, the decrease of MTR did not related to cognitive decline (230).

We noticed that we cannot exclude with certainty that the decrease of MTR values might have been determined by partial volume effects from the cerebrospinal fluid due to atrophy in

individuals with AD (220). Cerebrospinal fluid presents very low MTR measures, and the enlargement of the sulci could result in a decrease of MTR in voxels because of the combination of cortex and cerebrospinal fluid (220).
However, we do not believe that partial volume effects had a significant effect in our study because cortical volume was considered as a potential confounder in the analyses, and all MTR masks were adjusted by deleting voxels with high intensities in the co-registered pseudo-T2 map in order to avoid the contamination of cortical MTR measures with the cerebrospinal fluid (220).

Nonetheless, the histopathological alterations which are at the basis of the MTR reduction in the cortex in AD are still extensively unknown (220).
Reduced MTR metrics determined by focal myelin loss in the cortex were observed in subjects with multiple sclerosis (284), but it is unlikely that cortical demyelination is the cause of MTR reduction in the cortex of patients with AD (220).
A more probable hypothesis comes from a work of Patel et al. (285) who observed that dendritic branching is likely to be the principal element responsible for cortical MTR measurements, since the greater surface area of cellular membranes is associated with higher magnetization transfer between the free proton pool and the protons bound to macromolecules (285). The study showed that dendrites and their spines, in comparison to myelin, contribute 34-fold more exposure to the extracellular water, suggesting that cortical MTR measurements in the cortex are more sensitive to cellular membranes associated with dendrites than myelin (285). The loss of dendritic spines relates directly to synaptic dysfunction (220).
Individuals affected by AD present a relevant synaptic loss and their cognitive function correlate strongly with synaptic depletion (286).

A further possible cause for MTR reduction in AD brains is posttranslational modification of axonal proteins (220). This has been reported in an MTI study on postmortem brain slices, which evaluated blocks including microscopically non-lesioned brain tissue and micro-dissected adjacent tissue in order to determine the specific protein quantity (287). The authors observed that the reduction of MTR was generated by a hyperphosphorylation-related alteration in proton mobility. In accordance with these results, reduced cortical MTRs might be determined by hyperphosphorylation of proteins such as tau as well as pathologic protein deposition, an event that is known to be precedent to the cell death in AD and other neurodegenerative pathological entities (288).

In this context, a recent MTI study used a model-based multiparameter method in order to separately measure the amount of macromolecules and to explore the coupling features of protons (233). The authors found that this model-based MTI approach for the detection of macromolecules-related alterations in the mesial temporal lobe could increase the accuracy in distinguishing patients with AD from subjects with MCI, suggesting that MTI parameters might detect alterations in the macromolecular tissue composition determined by neurodegenerative disease (129).

The mentioned possible explanations are in line with the main finding of our work that shows that the decrease of cortical MTR in patients with AD is at least partly independent of cortical atrophy and that MTR reductions in the cortex explain cognitive decline beyond regional cortical volume (220).

Our work has several strong points. We investigated a large sample of patients with AD and of healthy older adults. The availability of a structural scan with high resolution permitted the segmentation of cortical and white matter compartments (220).

The cognitive function of AD patients was assessed not only with MMSE but also with the CERAD neuropsychological battery, which has been found to be valid and reliable measure of cognition in AD (289). Further, we assessed longitudinal clinical and radiological changes in the majority of our AD cohort.

Our study clearly has some limitations, such as the use of the NINCDS-ADRDA criteria, which have been introduced in 1984 for the diagnosis of AD (110). At the time of patient recruitment into this study, these criteria were routine. (220). Recently, the criteria have been updated, however, clinicopathological studies described that the 1984 NINCDS-ADRDA criteria provide a diagnostic sensitivity and specificity of 81% and 70%, respectively (112).

A further weakness of our work was that the spatial resolution of the MT sequence was limited, and therefore partial volume effects determined by cerebrospinal fluid in cortical regions cannot be excluded with certainty. However, these effects should be modest, and they have been taken into account in the image post-processing and in the statistical analyses by adjusting for volumetric measures (220).

Concerning the longitudinal analysis, we realize that the short follow-up time and the lack of longitudinal data from healthy control participants is an important limitation of our work.

In conclusion, in the course of this research project, we demonstrated that MTI is a reliable method for the quantification of microstructural tissue damage in AD. MTI might be very

attractive since it has high resolution and short acquisition time. Although these advantages, MTI has been scarcely explored in the study of the neurodegenerative diseases.

In our study, we investigated globally and regionally the microstructural changes, detected by MTI, in grey matter as well as white matter structures in a large cohort of patients with mild to moderate AD and in a group of age-matched non-demented elderly. Further, we explored the relationship between MTR values and cognitive function in AD. Finally, we investigated the amount of MTR changes over a mean observational time of 1.06 years and their relationship with cognitive decline in the majority of our AD cohort.

Among patients with AD, we found lower MTR values in the whole cortex, in the "AD-signature regions", in the NAWM as well as in the WMH. At the baseline, only MTRs in the cortex and in the AD signature regions related to cognitive impairment in AD. This association was modest, but independent of demographic data, atrophy or white matter damage. Over an observational time of 1.06 years, individuals with AD showed a significant MTR decline in the cortex only; however, MTR longitudinal changes were not associated with cognitive decline.

How AD- related pathophysiology might influence the mechanism of magnetization transfer in the grey matter is not fully understood (220). Although possible mechanisms responsible for MTR lowering in the cortex have been hypothesized, no histopathological studies have studied the association between MTI measures and cortical macromolecules. On the contrary, MTR reductions in the white matter have been associated mainly with demyelination and axonal loss in post-mortem studies with patients affected by multiple sclerosis.

So far, the contribution of MTI to the diagnosis of AD is still unknown, but our findings show that it supplies additional information apart from pure assessment of brain atrophy and visible white matter lesions (220). Post-mortem studies with AD patients might be helpful in the exploration of the histopathological basis that underlies MTR lowering in AD.

As alterations in the microscopic tissue composition may be present many years before visible changes and clinical symptom occur, longitudinal studies with individuals affected by MCI are needed to evaluate if MTR could improve the prediction of conversion to AD.

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