Characterization and validation of biomarkers for an improved classification of Alzheimer's disease pathology

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Karakterisering en validatie van biomarkers voor een verbeterde classificatie van de pathologie van de ziekte van Alzheimer

Proefschrift voorgelegd tot het behalen van de graad van doctor in de Biomedische Wetenschappen aan de Universiteit Antwerpen te verdedigen door

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Abbreviations

Aβ	amyloid-β
AD	Alzheimer's disease
ANOVA	analysis of variance
APP	amyloid precursor protein
APOE	apolipoprotein E
AUC	area under the curve
Aβ	β-amyloid
BACE1	β-site APP cleaving enzyme-1 or β-secretase
[^{11}C]PiB	[^{11}C]Pittsburgh Compound-B
CDR	clinical dementia rating
CH / CO	cognitively healthy
CI	confidence interval
CJD	Creutzfelt-Jakob disease
CSF	cerebrospinal fluid
CT	computed tomography
CV	coefficient of variation
DLB	dementia with Lewy bodies
DTI	diffusion tensor imaging
FABP3	fatty acid-binding protein 3
FDG / [^{18}F]FDG	[^{18}F]fluorodeoxyglucose
FTD	frontotemporal dementia
FTLD	frontotemporal lobar degeneration
GM	grey matter
IMR	immunomagnetic reduction
LP	lumbar puncture
MCI	mild cognitive impairment
MCI-AD	MCI progressing to AD dementia during clinical follow-up
MCI-nonAD	MCI progressing to another dementia disorder than AD during clinical follow-up
MMSE	mini-mental state examination
MRI	magnetic resonance imaging
MSD	Meso Scale Discovery
MXD	mixed dementia

NFL	neurofilament light
NFT	neurofibrillary tangle
Ng	neurogranin
Non-AD	dementia not due to Alzheimer's disease
NPV	negative predictive value
PET	positron emission tomography
PPV	positive predictive value
PrP^c	cellular prion protein
$pTau_{181}$	tau phosphorylated at threonine 181
$pTau_{231}$	tau phosphorylated at threonine 231
pTDP-43	TAR DNA-binding protein 43 phosphorylated at serine 409
RMSE	root-mean-square error
ROC	receiver operating characteristic
sAPP	soluble amyloid precursor protein
SCD	subjective cognitive decline
SD	standard deviation
Sens	sensitivity
Spec	specificity
Simoa	single-molecule array
SNAP	suspected non-AD pathology
SUVR	standardized uptake value ratio
TDP-43	TAR DNA-binding protein 43
tTau	total tau protein
VaD	vascular dementia or vascular disease
VBM	voxel-based morphometry
VLP1	visinin-like protein-1
WM	white matter

Summary

For many years, Alzheimer's disease (AD) could only be diagnosed in the presence of symptoms and by excluding other diseases. Nowadays, biomarkers are used to measure and confirm the presence of AD pathology during clinical disease and even before symptoms occur. Although the currently existing AD biomarkers have enabled a shift from a clinical exclusion to a biomarker-based diagnosis of AD over the past years, they still need further improvement as they are also changed (to a lesser extent) in non-AD disorders, do not always detect ongoing AD pathology, and have limited power to predict speed of clinical progression. As such, we aimed at an improved classification of AD pathology by the characterization and validation of existing and candidate biomarkers.

Biomarkers for AD are categorized according to the pathology they are supposed to represent: amyloid plaques (A), neurofibrillary tangles composed of hyperphosphorylated tau protein (T), and neurodegeneration (N). First we addressed the A category and showed that using Aβ isoforms, especially the $A\beta_{1-42}/A\beta_{1-40}$ ratio, in cerebrospinal fluid (CSF) as a measure of amyloid plaques increased diagnostic performance of CSF $A\beta_{1-42}$ alone and as such these isoforms are valuable additions to the A category. CSF BACE1 levels, however, did not show an added value in our pilot study as a measure of amyloid processing.

In a validation study of CSF $pTau_{181}$ in an autopsy-confirmed cohort as a measure of T we showed that $pTau_{181}$ had the highest diagnostic performance to differentiate between AD and non-AD and thus we confirmed that $pTau_{181}$ is an essential component of the AD CSF biomarker panel.

Regarding N biomarkers, we first performed a comparison of the predictive powers of the existing N biomarkers: CSF total tau, glucose metabolism on [^{18}F]FDG PET, and grey and white matter volume on magnetic resonance imaging using the ADNI database. In this study we found that the coexistence of grey and white matter atrophy is predictive of impending clinical decline in patients with mild cognitive impairment (MCI) presenting with amyloid and tau abnormalities. Whether the measurement of N could be improved by additional CSF biomarkers was assessed in

a following pilot study. The biomarkers that were tested (neurofilament light, neurogranin, visinin-like protein-1, YKL40, and fatty acid-binding protein 3) had all been shown to be predictive of cognitive decline in AD in previous studies. Although we were able to confirm some of these findings, we were not able to find any added value of these biomarkers to the core AD CSF biomarkers $A\beta_{1-42}$, $pTau_{181}$, and total tau. The exception is neurofilament light, which had an added value to predict progression to AD dementia of an heterogeneous group of MCI patients without both amyloid and tau abnormalities.

Improving the detection and classification of AD pathology will most probably benefit from combining biomarkers of different categories and modalities. Based on our studies, such a biomarker model should include the $A\beta_{1-42}/A\beta_{1-40}$ ratio and $pTau_{181}$ as CSF biomarkers of amyloid plaques and neurofibrillary pathology, respectively. The value of neurodegeneration biomarkers in a biomarker model might depend on whether a patient has abnormal levels of biomarkers of amyloid plaques and neurofibrillary pathology. Useful additions to a future biomarker model would be grey and white matter volume and CSF neurofilament light as measures of neurodegeneration. Consequently, further progress is needed to validate such a biomarker model with regard to its power to differentiate AD from non-AD disorders as well as to predict speed of clinical progression.

Samenvatting

In het verleden was het enkel mogelijk om de ziekte van Alzheimer te diagnosticeren wanneer er klinische symptomen aanwezig waren en door andere ziektes uit te sluiten. Tegenwoordig worden biomarkers gebruikt om de pathologie van de ziekte van Alzheimer in de hersenen te meten in patiënten met klinische symptomen, maar ook wanneer de symptomen nog niet zijn begonnen. De huidige biomarkers voor de ziekte van Alzheimer hebben een verschuiving mogelijk gemaakt van een diagnose gebaseerd op klinische exclusie naar één gebaseerd op metingen van de pathologie in de hersenen. Desondanks is verdere verbetering noodzakelijk, omdat de biomarkers ook veranderen in andere ziektes die dementie veroorzaken (weliswaar in mindere mate), ze niet altijd aanwezige pathologie van de ziekte van Alzheimer detecteren, en ze slechts in beperkte mate de snelheid van achteruitgang kunnen voorspellen. Wij beoogden dan ook een verbeterde classificatie van de pathologie van de ziekte van Alzheimer door karakterisering en validatie van bestaande en potentiële nieuwe biomarkers.

De bestaande biomarkers van de ziekte van Alzheimer worden gecategoriseerd naargelang de pathologie die ze vertegenwoordigen: amyloïde plaques (A), neurofibrillaire kluwens die bestaan uit hypergefosforyleerd tau eiwit (T) en neurodegeneratie (N). Allereerst richtten we ons op de A-categorie en toonden aan dat het meten van Aβ isovormen, met name de $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio, in het cerebrospinaal vocht (CSV) de diagnostische performantie vergroot in vergelijking met $A\beta_{1\text{-}42}$ alleen. Onze studie toonde dus aan dat Aβ isovormen een waardevolle toevoeging zijn aan de A-biomarkercategorie. We onderzochten ook BACE1 in CSV in een pilootproject, maar vonden geen waarde om BACE1 toe te voegen als biomarker aan de A-categorie.

Vervolgens toonden we in een validatiestudie in een autopsie-geconfirmeerde populatie dat $pTau_{181}$ in CSV de hoogste diagnostische performantie behaalde om de ziekte van Alzheimer te onderscheiden van andere ziektes die dementie veroorzaken. Bijgevolg bevestigden we dat $pTau_{181}$ een essentieel onderdeel is van de CSV biomarkers voor de ziekte van Alzheimer.

Tenslotte richtten we ons tot de biomarkers van neurodegeneratie, waarbij we eerst de voorspellende waarde van de bestaande N-biomarkers (totaal tau eiwit in CSV, glucosemetabolisme op [^{18}F]FDG-PET en grijze en witte stof volume op magnetische resonantie beeldvorming) vergeleken met behulp van de ADNI databank. Deze vergelijkende studie toonde aan dat de gezamenlijke atrofie van de grijze en witte stof nakende klinische achteruitgang voorspelt in patiënten met milde cognitieve klachten (*mild cognitive impairment*, MCI) die abnormale biomarkers hebben voor amyloïde plaques en neurofibrillaire kluwens. In een tweede neurodegeneratie biomarkerstudie, onderzochten we of potentieel nieuwe biomarkers de N-biomarkercategorie zouden versterken. Van de biomarkers die we testten (neurofilament light, neurogranin, visinin-like protein-1, YKL40 en fatty acid-binding protein 3) werd in het verleden aangetoond dat ze cognitieve achteruitgang in de ziekte van Alzheimer zouden kunnen voorspellen. Ondanks dat we een deel van die resultaten bevestigden, vonden we geen toegevoegde waarde voor de nieuwe biomarkers in vergelijking met de huidige CSV biomarkers $A\beta_{1\text{-}42}$, $pTau_{181}$ en totaal tau. Neurofilament light was echter een uitzondering, aangezien we voor die biomarker wel een toegevoegde waarde vonden om progressie naar dementie te voorspellen in MCI patiënten zonder abnormale biomarkers voor amyloïde plaques én neurofibrillaire kluwens.

De detectie en classificatie van de pathologie van de ziekte van Alzheimer zal hoogstwaarschijnlijk verbeterd worden door biomarkers uit verschillende categoriën en modaliteiten te combineren. Op basis van onze studies raden we aan om in een dergelijk biomarkermodel zeker de $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio en $pTau_{181}$ in CSV toe te voegen als biomarkers voor, respectievelijk, amyloïde plaques en neurofibrillaire kluwens. De waarde van de neurodegeneratie biomarkers hangt er mogelijks van af of een patiënt abnormale biomarkerwaarden heeft voor amyloïde plaques en neurofibrillaire kluwens. Grijze en witte stof volumes en neurofilament light zouden nuttige toevoegingen kunnen zijn als neurodegeneratie biomarkers aan een toekomstig biomarkermodel. Uitgebreide validatie van een dergelijk model is uiteraard noodzakelijk, met name met betrekking tot de performantie van het model om de ziekte van Alzheimer te onderscheiden van andere ziektes die dementie veroorzaken, alsook om snelheid van achteruitgang te voorspellen.

Chapter 1. Introduction

Alzheimer's disease

The proportion of older people in the world, especially in the Western world, increases as mortality falls and life expectancy increases. The most prevalent diseases affecting elderly are cancer, (cardio)vascular disease, and dementia. Dementia is detected in 5-7% of the world population over 60 years of age [1]. This figure is expected to increase as the world population ages.

The dementia syndrome is characterized by cognitive deterioration, emotional and affective changes as well as behavioral and personality changes, all having a negative impact on a person's functioning in daily life [2]. The most common cause of dementia at older age (>65 years) is Alzheimer's disease (AD), accounting for 60-80% of the affected cases, with its prevalence increasing exponentially with age. Other frequent causes are cerebrovascular disease, Lewy body disease, and frontotemporal lobar degeneration [3].

Dementia due to AD typically presents with episodic memory decline, often accompanied by disturbances of one or more other cognitive domains (i.e. executive functioning, orientation in time and space, or language) [4]. Atypical presentations of dementia due to AD are characterized by difficulties in language, visuospatial cognition, or executive functioning at an early stage, while memory decline usually sets in at a later time point [5].

The symptoms of AD have an insidious onset and are mild in the earliest phase of the disease. Patients then usually suffer from subjective cognitive decline, meaning they have cognitive complaints that cannot be detected objectively by a neuropsychological examination. When the complaints gradually worsen and can be objectively verified but do not yet affect the patient's performance of (instrumental) activities of daily living, the patient has reached the stage of mild cognitive impairment (MCI). Finally, the symptoms keep worsening progressively, affecting multiple cognitive domains, and hampering the patient in performing activities of daily living. The patient has then reached the dementia stage of AD. This gradual decline is described as the continuum of AD, which includes a preclinical stage, subjective cognitive decline, MCI, and dementia due to AD (Figure 1.1) [6].

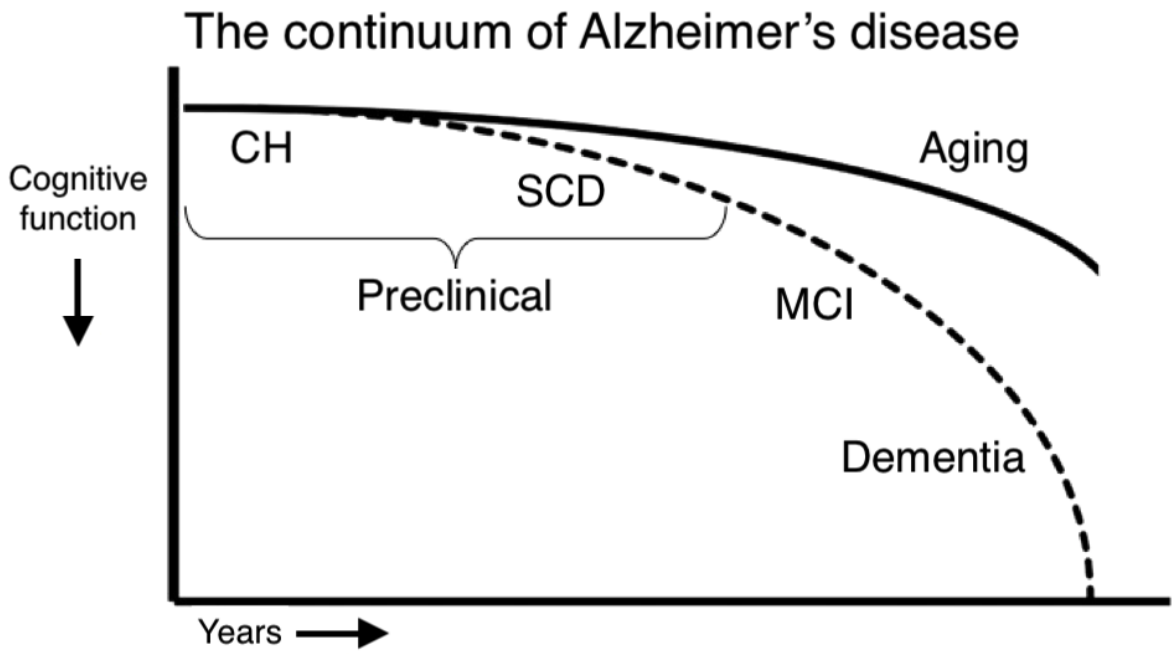

Figure 1.1 The disease continuum of AD (adapted from Sperling et al., 2011 [6]). Abbreviations: CH, cognitively healthy; MCI, mild cognitive impairment; SCD, subjective cognitive decline.

Besides cognitive decline, 35% to 85% of MCI and about 90% of dementia patients also present with behavioral and psychiatric symptoms [7, 8]. In the MCI stage, patients mostly suffer from depression, sleep disturbances, and anxiety. The emergence of apathy in MCI patients may be a sign of progression to dementia. In the dementia stage, the number of patients presenting with aggression, activity disturbances and psychoses increases [9].

The abovementioned symptoms of AD can also be caused by other diseases, such as major depression, cerebrovascular disease, a brain tumor, and central nervous system infections (e.g. neurosyphilis and neuroborreliosis). Structural brain imaging (i.e. magnetic resonance imaging (MRI) or computed tomography (CT)) is generally used to investigate cerebrovascular lesions and brain tumors, while blood and cerebrospinal fluid (CSF) tests can be performed in case of suspected central nervous system infections. An extensive neuropsychological examination is performed to evaluate which cognitive domains are affected and to what extent, and whether the patient also suffers from depression or other behavioral and psychiatric symptoms [10]. In the past, a diagnosis of AD could only be made based on clinical

symptoms and by excluding these other possible causes, which resulted at best in a diagnosis of probable AD [4].

As mentioned at the beginning of this section, dementia can also be caused by other diseases, of which cerebrovascular disease, Lewy body disease, and frontotemporal lobar degeneration are the most prevalent ones besides AD. However, although these diseases have characterizing typical symptoms and disease courses, these symptoms are not specific and show substantial overlap across different dementia disorders, making a differential diagnosis between AD and non-AD dementias challenging, especially in the early stages when symptoms are less overt. As such, specialized clinical centers achieve average sensitivity and specificity values of respectively 81% and 70% for a clinical diagnosis of probable AD [11]. The diagnostic accuracy is lower in the earliest stages of the disease and when the diagnostic work-up is performed in non-specialized centers [11].

In order to improve the diagnostic accuracy, great effort has been put into biological measures reflecting AD pathology, enabling clinicians to confirm the presence of AD pathology while the patient is alive.

Alzheimer's disease pathology: from local deposits to widespread neurodegeneration

The histopathological hallmarks of AD are extracellular amyloid-β (Aβ) protein deposits, and intracellular neurofibrillary pathology consisting of neuritic plaques, neurofibrillary tangles (NFT), and neuropil threads (Figure 1.2) [12-14]. Deposits of Aβ are first found in the neocortex (stage 1), followed by allocortical deposits (stage 2), deposits in diencephalic nuclei and the striatum (stage 3), in distinct brainstem nuclei (stage 4), and finally in the cerebellum and additional brainstem nuclei (stage 5) (Figure 1.3) [15]. Neurofibrillary lesions, on the other hand, first affect the transentorhinal region (stage I-II), followed by the limbic regions (stage III-IV), and finally by the isocortex and subcortical nuclei (stage V-VI) (Figure 1.4) [12]. It has even been suggested that sporadic AD tauopathy may begin in the lower brainstem with pretangle material in the locus coeruleus before affecting the transentorhinal region [16].

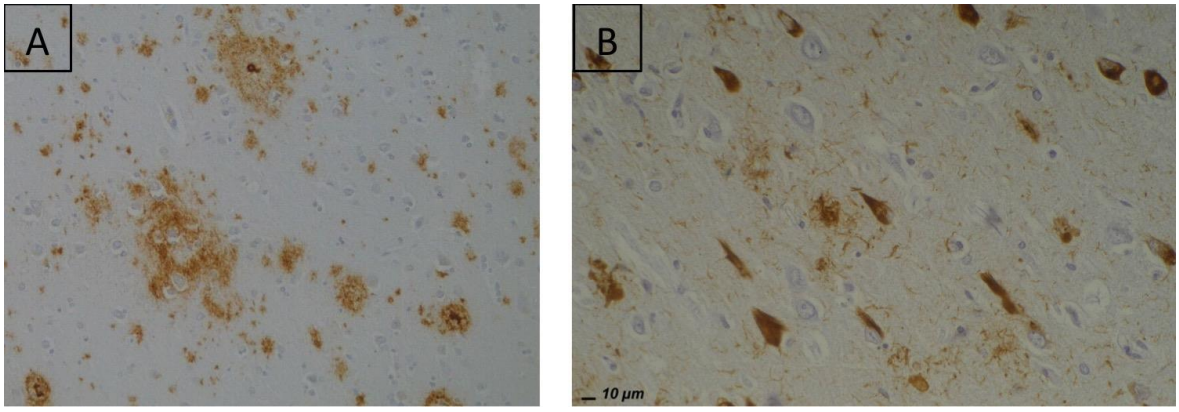

Figure 1.2 Immunohistochemical staining of (A) amyloid plaques, and (B) neurofibrillary tangles. (Courtesy of Institute Born-Bunge).

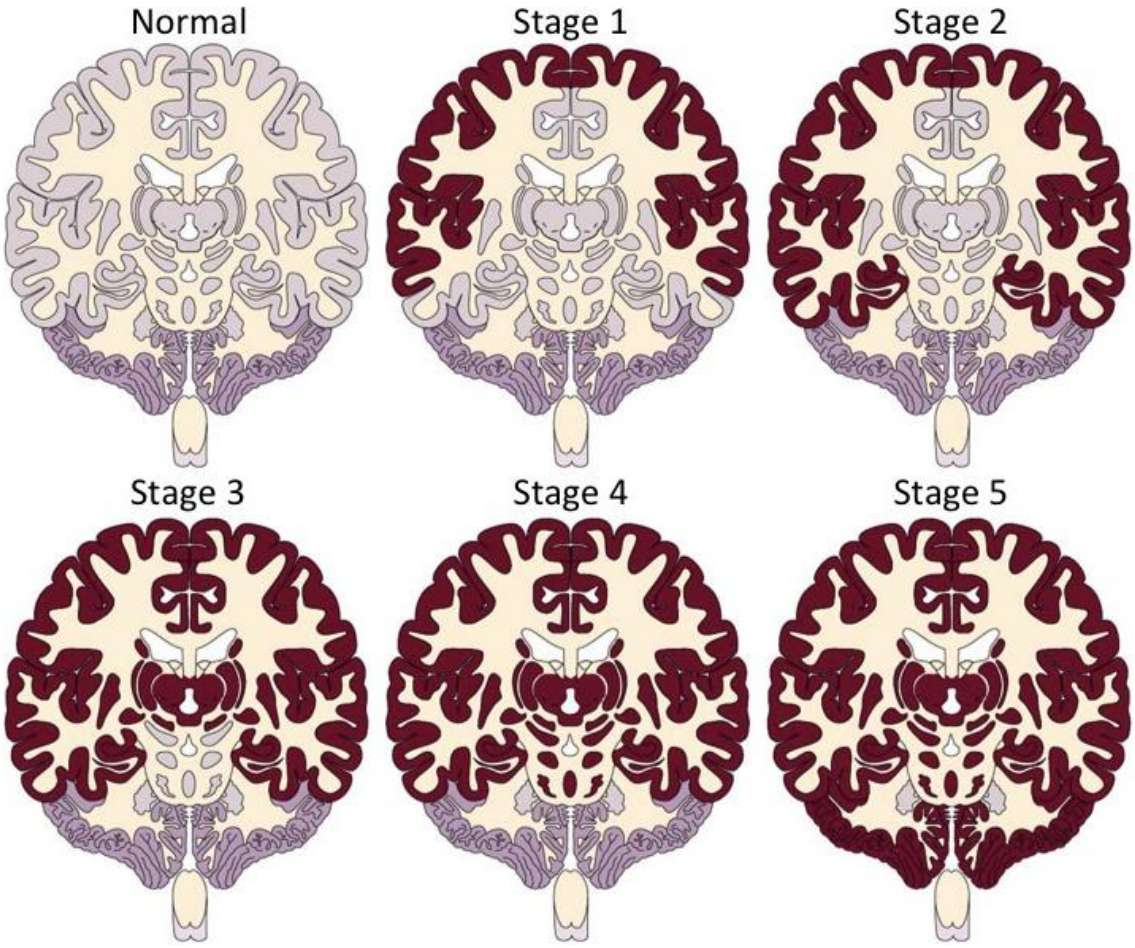

Figure 1.3 Staging of Aβ pathology in AD (adapted from Ovsepian and O'Leary, 2016 [17]).

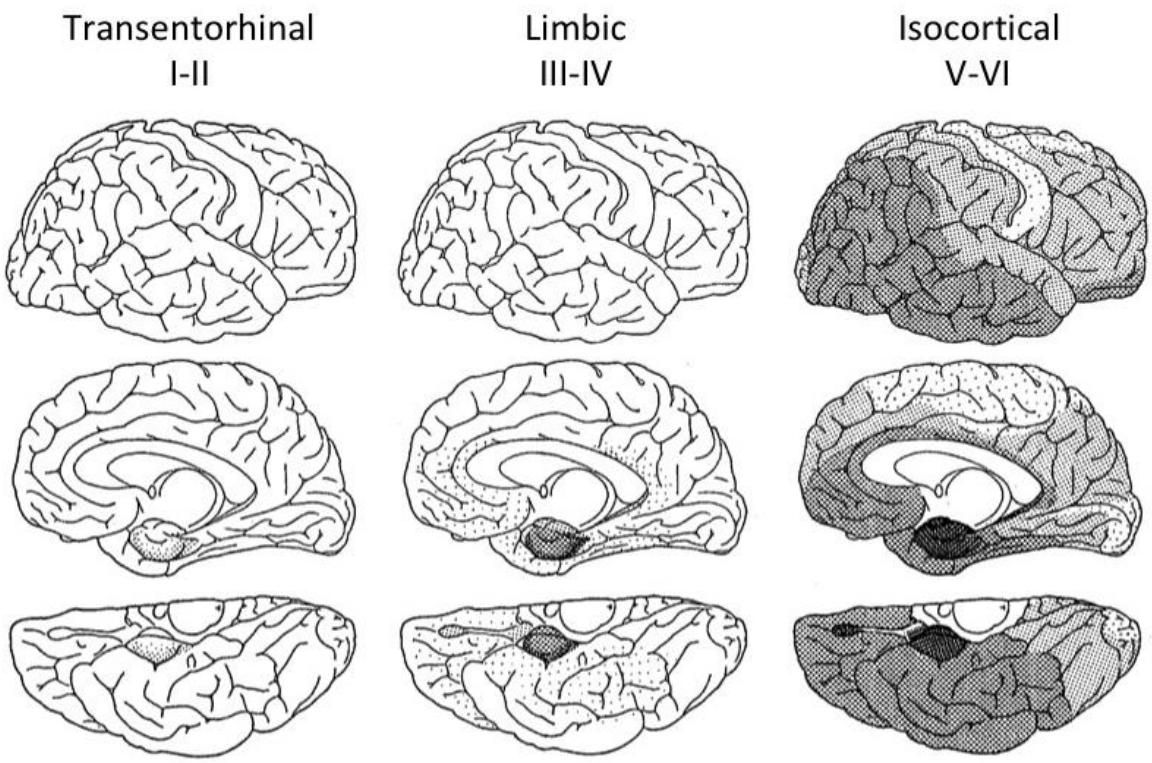

Figure 1.4 Staging of neurofibrillary changes in AD (adapted from Braak & Braak, 1991 [12]).

It is still debatable which pathological changes occur first, β-amyloidosis or tauopathy. According to the widely accepted amyloid-cascade hypothesis, AD initiates from an imbalance between production and clearance of the Aβ protein, resulting in an abundance of Aβ. In physiological conditions, Aβ is dissolved in the interstitial fluid. However, the abundance of Aβ leads to the formation of Aβ oligomers and fibrils, which eventually become insoluble and deposit into extracellular amyloid plaques [18].

Amyloid oligomers and plaques are toxic and impair neuronal function. Of the different Aβ species, the peptide of 42 amino acids ($A\beta_{1-42}$) is most toxic, probably due to its strong propensity to aggregate [19]. The toxic effects of Aβ include synaptic dysfunction, mitochondrial dysregulation, oxidative stress, and neuroinflammation [20].

It is often postulated that autosomal dominant (or familial) AD results from (relatively) increased production of aggregation-prone $A\beta_{1-42}$, while sporadic AD is probably more the result of reduced clearance of Aβ [18]. However, so far there is no

clear evidence of early-stage increased Aβ production, even in familial AD cases that were investigated up to 30 years before expected disease onset [21]. The same holds true for reduced clearance of Aβ in sporadic AD cases. Yet, most risk genes for AD somehow affect Aβ clearance, which points to reduced Aβ clearance as being part of the sporadic disease pathophysiology [22]. The most well-studied of these risk genes is *APOE* that encodes apoliprotein E, which is involved in the removal of Aβ. It has three common alleles: ε2, ε3, and ε4. The ε4 allele is associated with an increased risk of sporadic AD, with carriers of one allele and two alleles having a 3- and 12-fold higher risk than non-carriers, respectively [23]. Preclinical subjects possessing the ε4 allele show signs of β-amyloidosis almost a decade before non-carriers [24].

According to the amyloid-cascade hypothesis, the toxic effects of Aβ induce activity changes of kinases and phosphatases, leading to hyperphosphorylation of tau proteins. Hyperphosphorylated tau will, similarly to Aβ, form oligomers and fibrils, and finally aggregate into intracellular neurofibrillary tangles [25]. Especially oligomeric tau exerts toxic effects on neurons, also including synaptic dysfunction, mitochondrial and nuclear impairment, and microglial dysregulation [20].

Whether this hypothetical direct link between Aβ and the formation of neurofibrillary tangles exists, remains unclear. However, a recent study suggested that Aβ plaques are necessary albeit not sufficient to initiate the cascade of pathological tau spreading, because misfolded tau seeds with specific conformations are also required to trigger this process [26]. In that study, He and colleagues [26] support a mechanism whereby age-related neurofibrillary tangles developing in the medial temporal lobe independently of Aβ plaques [16] provide the initial source of pathological tau seeds that are unleashed by Aβ plaques to spread from the medial temporal lobe into neocortical regions.

Eventually, the combined toxicity of Aβ and tau will cause widespread degeneration of synapses and neurons [27, 28]. This entire process takes many years and starts decades (10-30 years) before the onset of clinical symptoms. Even after clinical symptoms come to surface, the pathological processes will continue to spread through the brain and symptoms will gradually become worse [12, 29].

Biomarkers to detect Alzheimer's disease pathology

As mentioned earlier, great effort has been put into biological measures, or so-called biomarkers, of AD pathology. After decades of research, seven biomarkers are now considered to be established as AD core pathological markers and are included in the biomarker-based research criteria for AD [5, 30-33]. They are divided into three categories (A/T/N), depending on the pathological process they represent.

First, biomarkers measuring amyloid deposition (A) are (1) decreased levels of $A\beta_{1-42}$ in the CSF, and (2) increased ligand retention of amyloid-specific probes on positron emission tomography (PET). Second, biomarkers of neurofibrillary tangles (tau, T) are (3) increased levels of phosphorylated tau in CSF, and (4) increased ligand retention of tau-specific probes on PET. Finally, general neuronal degeneration (N) can be measured by (5) increased CSF levels of total tau protein, (6) decreased glucose metabolism on [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) PET, and (7) brain atrophy on MRI [34].

Biomarker changes of amyloid deposition are detectable at an earlier stage than biomarker changes of neurofibrillary pathology (Figure 1.5) [35, 36]. However, that does not necessarily imply that amyloid deposition actually happens before neurofibrillary pathology. In fact, as mentioned in the previous section, amyloid deposition and neurofibrillary changes are both early and possibly simultaneous pathological events in AD. Although in-vivo biomarkers do reflect the specific pathophysiological processes they measure, they are less sensitive than histopathological assays after death [36]. As such, a possible explanation why amyloid biomarker changes are detected earlier than neurofibrillary biomarker changes is because amyloid pathology takes place outside the cells, as opposed to the initial intracellular location of neurofibrillary pathology, and might thus be more readily reflected in the CSF.

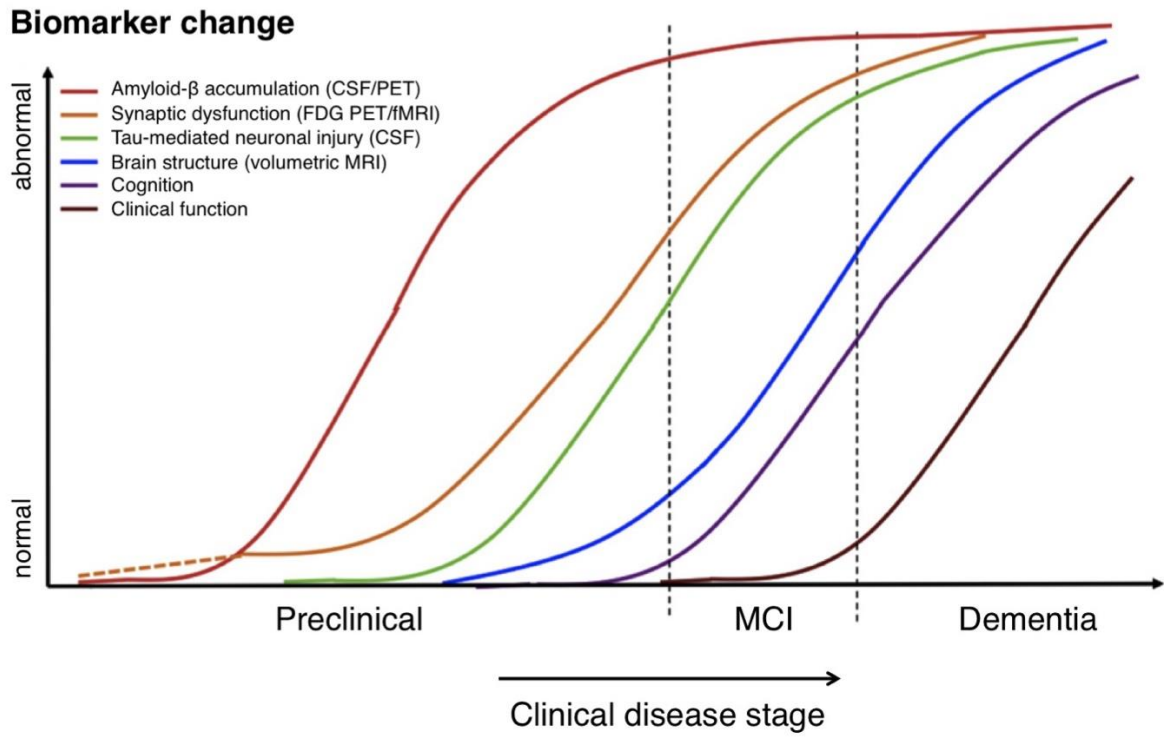

Figure 1.5 Hypothetical model of dynamic AD biomarker changes (adapted from Sperling et al., 2011 [6]). Abbreviations: CSF, cerebrospinal fluid; FDG, fluorodeoxyglucose; MCI, mild cognitive impairment; MRI, magnetic resonance imaging; PET, positron emission tomography.

The Aβ peptides are formed by sequential proteolytic cleavage of the amyloid precursor protein (APP) by β- and γ secretases, i.e. amyloidogenic processing of APP. The most abundant Aβ peptide found in the plaques is $A\beta_{1\text{-}42}$, which is highly hydrophobic in nature. Due to the extracellular aggregation of $A\beta_{1\text{-}42}$ into oligomers, fibrils, and insoluble plaques, the level of soluble monomeric $A\beta_{1\text{-}42}$ in the CSF decreases [37, 38]. Regarding tau, as it is an intracellular protein, its levels in CSF are low under physiological conditions. However, as neurons degenerate, they release their content into the surrounding space, leading to increased levels of total tau and phosphorylated tau in the CSF [38]. Pathological tau levels in CSF occur at a later stage of the disease process, closer to clinically detectable dementia [35, 36].

Synaptic and neuronal degeneration occur as an effect of and thus downstream of amyloid and neurofibrillary changes. Hence, the neurodegeneration biomarkers change later in the disease process, close to the onset of clinical symptoms (Figure 1.5) [35, 36].

As opposed to CSF biomarkers, imaging biomarkers provide additional topographical information. The amyloid plaque load detected by PET imitates the sequence of Aβ deposition found at autopsy [39]. The same holds true for tau pathology visualized by PET, as it also propagates through the brain as described by autopsy [12, 16]. Interestingly, the topographical tau distribution might be more relevant than its total amount, and more tightly associated with neurodegeneration and cognitive decline [40, 41]. It should be pointed out, however, that tau PET is the most recent addition to the AD biomarker panel and has thus not been studied as extensively as the other biomarkers. Therefore, contradictory results are still being reported regarding the clinical validity of tau PET [42-46].

The oldest AD imaging biomarkers are [^{18}F]FDG PET and MRI. Hypometabolism of glucose, measured on [^{18}F]FDG PET in parietal and temporal regions, results from the lower energy (i.e. glucose) demand in these regions where neurons are degenerating [47]. Atrophy on MRI is simply the loss of brain volume due to loss of neurons and associated cells. The first visually affected region is the medial temporal cortex, and particularly the hippocampus [48].

The clinical value of Alzheimer's disease biomarkers for diagnosis and prognosis

The main goal when using biomarkers is to confirm the presence of AD pathology while the patient is still alive, as opposed to merely excluding other diseases. In clinical practice, a neurologist will call in the aid of AD biomarkers for diagnostic or prognostic purposes. Diagnostic purposes include (1) increasing the diagnostic accuracy in case of suspected AD, (2) discriminating AD from other neurodegenerative and cerebrovascular brain disorders, (3) diagnosing AD in case of atypical presentations, and (4) diagnosing AD in its earliest stages (i.e. subjective cognitive decline and MCI). The latter purpose is closely related to prognosis, meaning to identify the early-stage patients that are likely to evolve to AD dementia as well as predict speed of progression [49]. It should be noted that it is recommended to routinely perform biomarker assessments in patients with early-onset dementia (<65 years) [50-52].

Regarding the use of biomarkers in case of suspected dementia due to AD, it has been shown that in case the full clinical diagnostic work-up (including imaging) leads to a diagnosis of probable AD dementia, there is no added value for CSF biomarkers or amyloid PET as they do not increase diagnostic accuracy in such cases [51, 53]. However, when the clinical diagnosis is doubtful, due to an atypical clinical course or etiologically mixed presentation, CSF biomarkers and amyloid PET have a clear added value and could aid in identifying the presence of underlying AD pathology [51, 53-55].

Atypical presentations of AD involve clinical symptoms closely related to non-AD diseases. However, with regard to pathology, they have the same fundamental pathological changes as typical AD, which can thus be identified by using the AD biomarkers [56, 57].

One of the difficulties in discriminating AD from other neurodegenerative and cerebrovascular brain disorders is the fact that several of these diseases present with AD-like pathology or mixed pathologies. This could lead to pathological biomarker values and possible misinterpretation of the biomarker results in the absence of clinical information. For instance, increased levels of CSF total tau caused by neuronal degeneration are also seen after ischemic stroke [58] and diseases with rapid and/or extensive neuronal degeneration, such as Creutzfeldt-Jakob disease [59]. As neurofibrillary tangles are more specific for AD, increased phosphorylated tau levels in CSF seem to be more specific for AD than total tau [60-62]. Regarding amyloid pathology, it has been shown that mixed pathologies are common, and for example 66%-72% of patients with dementia with Lewy bodies (DLB) also present with amyloid plaques [63, 64], which is associated with low CSF $A\beta_{1\text{-}42}$ concentrations [64] and increased ligand retention on amyloid PET in DLB patients [65]. However, it is uncertain whether amyloid pathology is inherent to DLB, or whether it is actually caused by mixed AD-DLB pathology [66].

Finally, atrophy and hypometabolism in regions typically affected in AD are the least specific for AD as they occur in multiple disorders [67]. Temporal atrophy, for instance, not only presents in AD but also in cerebrovascular disease, epilepsy,

anoxia, hippocampal sclerosis, TAR DNA-binding protein 43 (TDP-43)-opathy, primary age-related tauopathy [68], chronic traumatic encephalopathy, argyrophilic grain disease, progressive supranuclear palsy, and Pick's disease [67]. Temporoparietal hypometabolism is also found in primary progressive aphasia [69], corticobasal degeneration, and cerebrovascular disease [70].

In order to improve the discrimination between AD and non-AD dementias [60], new biomarkers that confirm a specific non-AD dementia type are needed.

Diagnostic and prognostic value of Alzheimer's disease biomarkers in the preclinical and MCI stages

Mild cognitive impairment is, just like dementia, a generic term used for cognitive impairment abnormal for age but not (yet) affecting a person's ability to function in daily life [71]. Although MCI is mainly caused by a neurodegenerative brain disease such as AD, vascular disease, frontotemporal lobar degeneration, Lewy body disease, etc., it can also result from other conditions such as trauma, depression, substance abuse, etc. [72]. Amnestic MCI patients (i.e. those with memory deficits) are at higher risk to progress to AD dementia compared with non-amnestic MCI patients, but not all will progress [73-76].

In order to identify MCI due to AD, also called prodromal AD [31, 33], the AD biomarkers are of immense importance to detect the specific pathological changes taking place (very) early in the disease process. Based on a meta-analysis, MCI subjects that later develop AD dementia have significantly lower CSF $A\beta_{1\text{-}42}$, and significantly increased CSF phosphorylated and total tau levels compared with those who do not progress [77]. Similarly, a European multicenter PET study has shown that the risk of progressing to AD dementia is higher in MCI subjects with a high amyloid plaque load as opposed to those with a normal plaque load [78]. Another meta-analysis showed that amyloid PET detects those MCI subjects progressing to AD dementia with a pooled sensitivity and specificity of 82% and 56%, respectively [79].

The sequential order of the AD biomarkers, from changes in biomarkers of amyloid pathology to neurofibrillary tangles and finally neurodegeneration (Figure 1.5),

renders biomarkers for amyloid plaques and neurofibrillary tangles more appropriate to detect AD pathology in the MCI and preclinical stages as opposed to the later-stage and less AD-specific neurodegenerative biomarkers. Indeed, it has been shown that synergy between amyloid changes and neurofibrillary tangles drive progression from MCI to dementia [80] and from the preclinical stage to MCI [81-85]. As such, low $A\beta_{1-42}$ and increased total and/or phosphorylated tau in the CSF are already found in the early MCI and subjective cognitive decline stages [86], while even being present in 28-36% of cognitively healthy individuals aged over 85 years [87, 88]. Furthermore, an increased amyloid plaque load is detected in 65% of cognitively healthy elderly aged over 80 years [89]. According to the amyloid-cascade hypothesis, the amyloid plaques start emerging at least 10 years and probably 20-30 years before the onset of clinical symptoms and they are as such regarded as a preclinical sign of AD and its biomarkers in CSF and PET as being useful for very early preclinical diagnosis [90]. It should be noted, however, that disease-modifying therapies are still lacking for AD. Biomarker-based diagnosis of preclinical AD should therefore only be performed in consented subjects within the context of research and clinical trials.

Regarding the neurodegeneration markers, they are not founded suitable to define preclinical AD. However, in the later MCI stage it was found that patients who progress to AD dementia present with hypometabolism in the typical AD affected areas on [^{18}F]FDG PET, whereas stable MCI patients show hypometabolism in other regions not suggestive for AD [91]. [^{18}F]FDG PET can detect progressive MCI subjects with a pooled sensitivity of 76% and specificity of 74% [79]. Finally, atrophy of the hippocampus and medial temporal lobe are found in MCI subjects progressing to AD [92]. Pooled sensitivity and specificity values of medial temporal atrophy to detect progressive MCI subjects are respectively 62% and 73%, while the values for hippocampal atrophy are 60% and 75% [79].

Although the biomarkers of neurodegeneration can predict progression of MCI to dementia, their added value compared with amyloid and neurofibrillary tangles synergy is still unclear. It is expected, however, that neurodegeneration markers are more predictive of the speed of progression in general, without being specific for AD [34].

Need for improved biomarkers for the classification of Alzheimer's disease pathology

Due to clinical overlap between AD and non-AD disorders [93], biomarkers are needed to detect or verify pathology in the brain and to improve differential diagnosis. So far, biomarkers of AD pathology have been most successful. Yet, the currently existing AD biomarkers are also changed, albeit to a lesser extent, in non-AD disorders and do not always detect ongoing AD pathology due to limited sensitivity, which reduces their early and differential diagnostic power.

On one hand, biomarker-based differential diagnosis could be improved by biomarkers specific for non-AD pathologies. Examples are specific measures of Lewy bodies found in Lewy body disease, and TDP-43 inclusions in frontotemporal lobar degeneration. On the other hand, improving the specificity of the biomarkers for AD pathology, for example by including levels of different isoforms of a biomarker, using a more specific PET ligand, investigating the inter-relationship between the biomarkers, etc., might also enhance differential and early diagnosis and prognosis.

This PhD work has mainly focused on improving the accuracy of early and differential diagnosis of AD by profound characterization of the existing core AD biomarkers and proposed AD biomarker candidates. As this research was supported as an IWT/VLAIO Baekeland mandate, it was performed in close collaboration with two spinoff companies of the University of Antwerp and KU Leuven: ico**metrix** and ADx NeuroSciences. As such, the research focused on the characterization and validation of existing and novel CSF biomarkers for AD pathology as well as of the value of the ico**brain** pipeline to measure brain volumes on MRI. Due to this Baekeland collaboration, this PhD work was very applied to biomarkers in clinical practice.

Chapter 2. Aims and objectives

Within this IWT/VLAIO Baekeland collaboration with ico**metrix** and ADx NeuroSciences we aimed to validate existing biomarkers as well as characterize novel biomarkers to enhance the detection of underlying pathology and thus improve diagnosis and prognosis of early stages of AD. Therefore, the general aim of this project was to perform (1) characterization studies of novel biomarkers for amyloid plaque pathology, (2) a validation study of the established CSF biomarker for neurofibrillary tangles (i.e. tau phosphorylated at threonine 181 ($pTau_{181}$)), (3) a comparison of the prognostic powers of the established neurodegeneration biomarkers, and (4) a characterization study of a panel of possible novel CSF biomarkers of neurodegeneration. The specific research objectives were as follows:

The first objective was to assess the potential diagnostic accuracy of Aβ isoforms in CSF for differential dementia diagnoses as well as for early AD diagnosis. In addition, in order to evaluate the added value of the Aβ isoforms, their diagnostic values were compared with the diagnostic values of CSF $A\beta_{1-42}$, total tau and $pTau_{181}$.

The second objective was to investigate the prognostic power of β-site amyloid precursor protein cleaving enzyme, the enzyme cleaving APP to form Aβ peptides, in CSF to predict cognitive decline in MCI patients.

The third objective was to investigate the value of $pTau_{181}$ in the AD CSF biomarker panel for differential dementia diagnosis in autopsy confirmed AD and non-AD patients.

The fourth objective was to perform a head-to-head comparison of the prognostic values of the currently established neurodegeneration biomarkers, CSF total tau, hypometabolism on [^{18}F]FDG PET, and brain atrophy on MRI, to predict progression from MCI to dementia in four years time.

The fifth and final objective was to investigate the prognostic power of possible new CSF neurodegeneration markers, neurogranin, visinin-like protein-1, neurofilament light, fatty acid-binding protein 3, and YKL40 (also known as chitinase 3-like 1) to predict cognitive decline in MCI patients.

Chapter 3. Amyloid plaque pathology

3.1 Diagnostic accuracy of cerebrospinal fluid amyloid-β isoforms for early and differential dementia diagnosis

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Abstract

Overlapping CSF levels between AD and non-AD patients decrease differential diagnostic accuracy of the AD core CSF biomarkers. Aβ isoforms might improve the AD versus non-AD differential diagnosis. Here, we aimed to determine the added diagnostic value of Aβ isoforms $Aβ_{1-37}$, $Aβ_{1-38}$ and $Aβ_{1-40}$ as compared to the AD CSF biomarkers $Aβ_{1-42}$, tTau and $pTau_{181}$.

CSF from patients with dementia due to AD (n=50), non-AD dementias (n=50), MCI due to AD (n=50) and non-demented controls (n=50) was analyzed with a prototype multiplex assay using MSD detection technology. The non-AD group consisted of frontotemporal dementia (n=17), dementia with Lewy bodies (n=17) and vascular dementia (n=16).

$Aβ_{1-37}$ and $Aβ_{1-38}$ increased accuracy to differentiate AD from frontotemporal dementia or dementia with Lewy bodies. $Aβ_{1-37}$, $Aβ_{1-38}$ and $Aβ_{1-40}$ levels correlated with Mini-Mental State Examination scores and disease duration in dementia due to AD. The $Aβ_{1-42}/Aβ_{1-40}$ ratio improved diagnostic performance of $Aβ_{1-42}$ in most differential diagnostic situations. $Aβ_{1-42}$ levels were lower in *APOE* ε4 carriers compared to non-carriers.

In conclusion, Aβ isoforms help to differentiate AD from frontotemporal dementia and dementia with Lewy bodies. Aβ isoforms increase diagnostic performance of $Aβ_{1-42}$. In contrast to $Aβ_{1-42}$, Aβ isoforms seem to be correlated with disease severity in AD. Adding the Aβ isoforms to the current biomarker panel could enhance diagnostic accuracy.

Introduction

Amyloid plaques, one of the major neuropathological hallmarks of AD, mainly consist of aggregates of carboxyterminally elongated forms of Aβ peptides [94], resulting from cleavage of the transmembrane APP by β- and γ-secretase [37]. The most abundant Aβ peptides in CSF are $Aβ_{1-38}$, $Aβ_{1-40}$ and $Aβ_{1-42}$ [95], of which $Aβ_{1-42}$ is the most pathological in AD as it is most prone to aggregation into Aβ plaques [96].

The combined assessment of CSF $Aβ_{1-42}$, total tau protein (tTau) and tau phosphorylated at threonine 181 ($pTau_{181}$) increases diagnostic certainty for AD [97]. Compared with controls, the AD CSF biomarker profile consists of decreased $Aβ_{1-42}$ and increased tTau and/or $pTau_{181}$ concentrations. However, when compared with non-AD dementias, these differences are less pronounced as the concentrations in patients with non-AD dementias are generally intermediate between those found in controls and AD patients, indicating an overlap between AD and non-AD patients [98].

Determining CSF Aβ isoforms might improve the AD versus non-AD differential diagnosis, as some evidence exists that $Aβ_{1-42}/Aβ_{1-40}$ or $Aβ_{1-42}/Aβ_{1-38}$ ratios improve discriminating AD from non-AD dementias in comparison to $Aβ_{1-42}$ alone [99, 100]. Indeed, several studies have shown the CSF levels of $Aβ_{1-38}$ are decreased in frontotemporal dementia (FTD) compared with AD and non-demented controls [101, 102]. Using the $Aβ_{1-42}/Aβ_{1-38}$ ratio FTD could be differentiated from AD with a sensitivity and specificity of 82% [101]. As AD pathology is common in dementia with Lewy bodies (DLB) and the presence of senile plaques in DLB patients is associated with low CSF $Aβ_{1-42}$ concentrations, the determination of CSF $Aβ_{1-42}$ levels is of limited value for discriminating AD and DLB [64]. However, it has been shown that the ratios of $Aβ_{1-42}/Aβ_{1-37}$ and $Aβ_{1-42}/Aβ_{1-38}$ can differentiate between AD and DLB [103, 104].

In this study the Aβ isoforms $Aβ_{1-37}$, $Aβ_{1-38}$, $Aβ_{1-40}$ and $Aβ_{1-42}$ were analyzed and four research questions were explored: 1) Do Aβ isoforms correlate with disease severity in AD? 2) Do the Aβ isoforms levels differ between apolipoprotein E (*APOE*) ε4 carriers and non-carriers? 3) Does the ratio of $Aβ_{1-42}/Aβ_{1-40}$ increase the diagnostic

performance of $A\beta_{1-42}$ alone? 4) What is the added diagnostic value of the Aβ isoforms?

The potential diagnostic accuracy of the Aβ peptides $A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-40}$ and $A\beta_{1-42}$ was assessed for differential dementia diagnoses as well as for early AD diagnosis. In addition, in order to evaluate the added value of the Aβ isoforms, their diagnostic values were compared with the diagnostic values of $A\beta_{1-42}$, tTau and $pTau_{181}$.

Materials and methods

Study population

Samples from patients and controls were selected from the Biobank of the Institute Born-Bunge. Only samples from patients recruited in the Memory Clinic and Department of Neurology of Hospital Network Antwerp (ZNA) were selected to avoid inter-center variability due to possible differences in pre-analytical steps. Patients with dementia due to AD (n=50), MCI due to AD (n=50) and patients with non-AD dementias (n=50) were included. The non-AD group consisted of 17 patients with FTD, 17 DLB patients and 16 patients with vascular dementia (VaD).

Patients with MCI and dementia due to AD were diagnosed according to the NIA-AA criteria [5, 32], with at least intermediate probability of AD etiology (based on the CSF biomarkers or hippocampal volume on MRI). MCI due to AD and dementia due to AD will hereafter be referred to as 'MCI' and 'AD' respectively. FTD, DLB and VaD were diagnosed according to the criteria described by Neary et al. [105], the clinical diagnostic criteria of McKeith et al. [106] and the NINDS-AIREN criteria [107] respectively.

The control group consisted of cognitively healthy elderly (n=35) in whom cognitive deterioration was ruled out by means of neuropsychological screening. Cognitively healthy elderly also fulfilled the following inclusion criteria: (1) no neurological or psychiatric antecedents and (2) no central nervous system disease following extensive clinical examination. The control group also consisted of patients with neurological diseases in whom neurodegenerative disorders were ruled out by means of an extensive neurological work-up (n=15). The study was approved by the

local ethics committee (University Hospital Antwerp) and all subjects gave their written informed consent.

CSF sampling

Lumbar puncture (LP), CSF sampling and handling have been performed according to a standard protocol [53]. CSF samples were stored at -80°C until analysis.

CSF biomarker analyses

CSF biomarker analyses of $A\beta_{1-42}$, tTau and $pTau_{181}$ were performed using commercially available single parameter ELISA kits (INNOTEST®, Fujirebio Europe, Ghent, Belgium) at the BIODEM lab as previously described [108]. CSF biomarker analyses of $A\beta_{1-37}$, $A\beta_{1-38}$ and $A\beta_{1-40}$ were performed at QPS Netherlands BV (Groningen, The Netherlands) with a prototype multiplex assay developed by Janssen Research and Development that uses Meso Scale Discovery (MSD) detection technology as previously described [109].

Briefly, the multiplex assay involved a sandwich immunoassay with electrochemoluminescence detection. Standards of human $A\beta_{1-37}$, $A\beta_{1-38}$ and $A\beta_{1-40}$ (AnaSpec, San Jose, USA) were dissolved in dimethylsulphoxide at 0.1 mg/mL and stored at -80°C. For use in the assay, peptides were further diluted in casein buffer (0.1% casein in PBS). Purified monoclonal antibodies specific for $A\beta_{1-37}$ (JRD/Aβ37/3), $A\beta_{1-38}$ (J&JPRD/Aβ38/5) and $A\beta_{1-40}$ (JRF/cAβ40/28) were coated on MSD 4-plex 96-well plates on spatially distinct spots. Plates were blocked with casein buffer for 1-4 h at room temperature. After washing, standards, quality control samples and 1/2 prediluted CSF samples were incubated overnight at 4°C together with MSD SULFO-TAG™-labeled human-specific detection antibody JRF/AβN/25. JRF/AβN/25 detects an end-specific epitope of Aβ leading to the detection of full-length Aβ peptides ($A\beta_{1-x}$). After overnight incubation, plates were washed, after which 2x Read Buffer (MSD) was added according to the manufacturer's recommendations and plates were read on MSD Sector Imager 6000. $A\beta_{1-37}$, $A\beta_{1-38}$ and $A\beta_{1-40}$ concentrations were determined by interpolation from the standard curve using MSD Workbench software and 4 parameter logistic model with $1/Y^2$ weighting function. All calibration standards and CSF samples were analyzed in

duplicate. Only mean values with a replicate well coefficient of variation (CV) of less than or equal to 20.0% were accepted. The samples of the different diagnostic groups were tested randomized over multiple plates. The means for the interplate CV for the quality control samples were less than 12% for all analytes. The upper and lower limit of quantification, determined as the highest and lowest calibrator concentration for which overall CV and bias were ≤25.0%, was 4.57 pg/mL and 10 000 pg/mL respectively for all measured Aβ peptides.

Disease severity in AD

Disease severity of AD was estimated by Mini-Mental State Examination (MMSE) scores and disease duration. MMSE tests were always performed 3 months before or after LP. If available, the yearly change in MMSE, i.e. the difference between the earliest MMSE score and the most recent one divided by their time interval, was also reported. Disease duration was considered as the difference between age at onset and age at LP.

APOE genotyping

The isolation of genomic DNA from peripheral blood lymphocytes was performed at the Genetic Service Facility (http://www.vibgeneticservicefacility.be) of the VIB Department of Molecular Genetics on a Magtration® System 8Lx. robotic platform. SNPs in *APOE* (rs429358 and rs7412, determining the ε2/ε3/ε4 polymorphism) were genotyped by Sanger sequencing.

Statistical analyses

Statistical analyses were performed using SPSS 20. First, a Kolmogorov-Smirnov test was performed to check for normal distribution. Since most variables did not follow a normal distribution, non-parametric tests were used. To compare gender distribution and *APOE* carrier status across the groups a Chi-square test was performed. A Kruskal-Wallis test was used to compare biomarker data over all groups. Subsequently, Mann-Whitney U tests were performed to compare groups separately. To assess correlations, Spearman's Rho correlation tests were performed. Receiver operating characteristic (ROC) curve analyses were used to

obtain area under the curve (AUC) values and to define optimal cut-off values to discriminate MCI and AD from all other groups. The cut-off values were determined by calculating the maximal sum of sensitivity and specificity (i.e. maximizing the Youden index). In order to compare AUC values DeLong tests were performed by using the pROC package [110] in the statistical software package R (R Core Team). Correction for multiple testing was not performed due to the small study population and the explorative nature of this study.

Results

Study population: demographic, clinical and biomarker data

One AD patient and one MCI patient were excluded from statistical analyses because all their Aβ isoforms concentrations were below the lowest range. The groups were not age and gender matched. Table 3.1 summarizes demographic, clinical and biomarker data for all groups.

Correlation with MMSE

In the MCI group none of the correlations were significant (Table 3.2). However, in the AD group $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ correlated moderately with MMSE scores. Yearly change in MMSE correlated significantly but weakly (P<.05) with $A\beta_{1\text{-}42}$ in the MCI group.

Correlation with disease duration

In the AD population the correlations of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ with disease duration were weak but significant (Table 3.2).

Effect of APOE ε4

The Aβ isoforms levels were compared between subjects carrying one or two ε4 alleles (n=58) and non-carriers (n=86) (Table 3.3). $A\beta_{1\text{-}42}$ was significantly lower in ε4 carriers (P<.001), while $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ were not significantly different.

In the MCI and AD populations separately none of the biomarkers differed significantly between ε4 carriers and non-carriers. However, when combining both

diagnostic groups, $A\beta_{1-42}$ was significantly lower in carriers than non-carriers (P<.05). This was also found in the non-AD group (P<.05).

Diagnostic accuracy

The ROC curve analysis results of the best performing biomarkers are summarized in Table 3.4, while the remaining data are given in the Supplementary Material.

AD versus MCI

$A\beta_{1-42}$, tTau and $pTau_{181}$ did not differentiate between MCI and AD, keeping in mind these analytes were used to define these groups. The AUC values of the Aβ isoforms were below 0.800 (Supplementary table 3.1).

AD and MCI versus controls

The biomarkers performing best when comparing AD patients and controls were $A\beta_{1-42}$/tTau and $A\beta_{1-42}/pTau_{181}$. $A\beta_{1-42}/A\beta_{1-40}$ performed comparably to $A\beta_{1-42}$ for discriminating AD from controls (Table 3.5; Supplementary table 3.2).

The biomarkers performing best when comparing MCI patients and controls were $A\beta_{1-42}$/tTau and $A\beta_{1-42}/A\beta_{1-40}$. $A\beta_{1-42}/A\beta_{1-40}$ as well as $A\beta_{1-42}/A\beta_{1-37}$ significantly increased the performance of $A\beta_{1-42}$ alone to discriminate MCI and controls (Table 3.5; Supplementary table 3.2).

AD and MCI versus non-AD

The best performing biomarkers when comparing AD patients and non-AD dementias were the $A\beta_{1-42}$/tTau and $A\beta_{1-42}/pTau_{181}$ ratios. The AUC values of the $A\beta_{1-42}/A\beta_{1-38}$ and $A\beta_{1-42}/A\beta_{1-37}$ ratios reached the 0.800 threshold and were significantly higher than the AUC of $A\beta_{1-42}$ alone (Table 3.5; Supplementary table 3.3).

When comparing MCI with non-AD dementia patients, the best performing biomarkers were $pTau_{181}$ and $A\beta_{1-42}/A\beta_{1-40}$. $A\beta_{1-42}/A\beta_{1-38}$ and $A\beta_{1-42}/A\beta_{1-37}$ also significantly increased the power of $A\beta_{1-42}$ to discriminate between MCI and non-AD (Table 3.5; Supplementary table 3.3).

AD and MCI versus FTD

The best biomarkers to distinguish AD and FTD were $A\beta_{1-42}/A\beta_{1-37}$ and the relative value of $A\beta_{1-42}$, i.e. the ratio of $A\beta_{1-42}$ to the sum of all Aβ isoforms (Supplementary table 3.4). All ratios increased the performance of $A\beta_{1-42}$ significantly (Table 3.5).

The $A\beta_{1-42}/A\beta_{1-40}$ ratio was the best biomarker to distinguish MCI and FTD. $A\beta_{1-40}$, $A\beta_{1-42}/A\beta_{1-38}$ and $A\beta_{1-42}/A\beta_{1-37}$ also performed well, with all ratios significantly increasing the performance of $A\beta_{1-42}$ (Table 3.5; Supplementary table 3.4).

AD and MCI versus VaD

The best biomarkers to distinguish AD and VaD were $A\beta_{1-42}$/tTau and $A\beta_{1-42}/pTau_{181}$, comparable to the AD versus controls situation. The diagnostic accuracy of $A\beta_{1-42}$ was not increased by ratios with the other Aβ isoforms (Table 3.5; Supplementary table 3.5).

The best performing biomarker when differentiating MCI and VaD was $pTau_{181}$. The $A\beta_{1-42}/A\beta_{1-40}$ ratio increased the diagnostic accuracy of $A\beta_{1-42}$ significantly (Table 3.5; Supplementary table 3.5).

AD and MCI versus DLB

The best biomarkers to differentiate AD between DLB were $A\beta_{1-42}/A\beta_{1-38}$ and $A\beta_{1-42}$/tTau (Supplementary table 3.6). Similar performances were found for tTau, $A\beta_{1-42}/A\beta_{1-37}$, $pTau_{181}$ and $A\beta_{1-38}/A\beta_{1-40}$. The diagnostic accuracy of $A\beta_{1-42}$ was not increased by any isoform ratio (Table 3.5).

The best performing biomarkers to differentiate MCI and DLB were $pTau_{181}$ and $A\beta_{1-38}$. The $A\beta_{1-42}/A\beta_{1-38}$ ratio and $A\beta_{1-37}$ performed similarly. The performance of $A\beta_{1-42}$ was substantially increased by the ratios with the other Aβ isoforms (Table 3.5; Supplementary table 3.6).

Discussion

This study was set up to investigate the potential diagnostic value of the Aβ peptides $A\beta_{1-37}$, $A\beta_{1-38}$ and $A\beta_{1-40}$ for differential dementia diagnosis as well as for early AD

diagnosis. In addition, in order to evaluate the added value of the Aβ isoforms, their diagnostic values were compared with the diagnostic values of $A\beta_{1-42}$, tTau and $pTau_{181}$.

The four research questions posed in this study will be further discussed in this section. The research questions regarding diagnostic performance of $A\beta_{1-42}/A\beta_{1-40}$ and with regard to the added value of the Aβ isoforms are combined in the subsection 'Diagnostic performance' as they largely coincide.

Correlation with disease severity in AD

When assessing the correlation of the Aβ isoforms as well as the $A\beta_{1-42}/A\beta_{1-40}$ ratio with MMSE scores and disease duration, significant weak to moderate correlations were found in the AD population, except for the $A\beta_{1-42}/A\beta_{1-40}$ ratio. On the other hand, no significant correlations in the MCI population were found. Similar results were found by Mulugeta et al. [104], though they had to combine all investigated patients in order to find significant correlations. Our results imply there might be a correlation of the Aβ isoforms with disease severity in AD and the Aβ isoforms could have a prognostic value in AD. However, this needs further investigation in larger, independent cohorts before any conclusions can be drawn.

Difference between APOE ε4 carriers and non-carriers

The Aβ isoforms $A\beta_{1-37}$, $A\beta_{1-38}$ and $A\beta_{1-40}$ were not different between ε4 carriers and non-carriers. The levels of $A\beta_{1-42}$ were always lower in ε4 carriers compared with non-carriers, although this difference was not always significant. In the AD and MCI groups separately, none of the biomarkers were significantly different between carriers and non-carriers. However, when combining both AD and MCI groups, there was a significant difference in the level of $A\beta_{1-42}$ between carriers and non-carriers, which could be a confirmation of results found in a study on autopsy-confirmed AD patients [111]. This change in significance could be caused by the higher power when combining both groups. In the pooled non-AD population a significant difference was found in levels of $A\beta_{1-42}$. This difference might be explained by the fact that ε4 is a risk factor for AD co-pathology in the brain of non-AD dementias too [64].

Diagnostic performance

$A\beta_{1-42}$, tTau and $pTau_{181}$ did not differentiate between MCI and AD. This was to be expected, since both groups have AD and these biomarkers have almost reached their maximal increase or decrease in MCI, only changing minimally with disease evolution as from the MCI stage. Interestingly, comparable differences were found regarding the Aβ isoforms when comparing MCI and AD with controls and the non-AD groups. This once more points to the common AD pathophysiology in MCI and AD groups. Based on the ROC analyses, the Aβ isoforms were able to differentiate between MCI and AD groups, although the AUC values were below 0.800. This might be explained by the moderate correlation of the Aβ isoforms with disease severity. Both results might point to changes of these isoforms with AD progression, in contrast to $A\beta_{1-42}$ that remains stable.

When comparing MCI and AD and controls, analyzing Aβ isoforms has an added value, as $A\beta_{1-42}/A\beta_{1-40}$ performed slightly better compared with $A\beta_{1-42}$ for discriminating AD from controls and substantially better for discriminating MCI and controls. However, since the AUC value of $A\beta_{1-42}/A\beta_{1-40}$ is comparable or lower than those of $A\beta_{1-42}$/tTau and $A\beta_{1-42}/pTau_{181}$, the added diagnostic value of the Aβ isoforms is considered to be limited.

According to our in-house validated $A\beta_{1-42}$ cut-off to discriminate AD from cognitively healthy elderly (638.5 pg/mL), five patients had normal $A\beta_{1-42}$ levels. However, their $A\beta_{1-42}/A\beta_{1-40}$ ratio was decreased compared with controls, although this difference was not significant (P>.05), probably due to the small number of patients. We hypothesize that the $A\beta_{1-42}/A\beta_{1-40}$ ratio has a diagnostic value in AD patients having normal values of $A\beta_{1-42}$, since the $A\beta_{1-42}/A\beta_{1-40}$ ratio is decreased in these patients compared with controls [112, 113], which should be further investigated in larger cohorts.

Given our results for AD versus FTD, analyzing $A\beta_{1-37}$ has an added diagnostic value. It should also be noted our results for $A\beta_{1-38}$ are comparable to those of Gabelle et al. [102]. However, in contrast to Gabelle et al. [102], we found no added value of $A\beta_{1-38}$ for the differential diagnosis of AD and FTD given the relatively low AUC (not

exceeding 0.800). In addition, we found similar sensitivity but lower specificity values for $A\beta_{1-42}/A\beta_{1-38}$ as Bibl et al. [101] for discriminating AD and FTD.

To differentiate AD and VaD, the core AD biomarkers performed best. The $A\beta_{1-42}/A\beta_{1-40}$ ratio increased the diagnostic accuracy of $A\beta_{1-42}$ alone, pointing to a diagnostic value of the Aβ isoforms. However, since the AUC values were not higher than those of $A\beta_{1-42}$/tTau and $A\beta_{1-42}/pTau_{181}$ in the AD versus VaD situation and $pTau_{181}$ and $A\beta_{1-42}/pTau_{181}$ in the MCI versus VaD situation, the added diagnostic value is limited.

As the AUC value of $A\beta_{1-42}/A\beta_{1-38}$ when comparing AD and DLB was only a little higher than the AUC value of $A\beta_{1-42}$/tTau, the added diagnostic value of $A\beta_{1-38}$ is only limited. This also held true for MCI and DLB, as the best performing biomarkers $pTau_{181}$ and $A\beta_{1-38}$ had equal AUC values. The performance of $A\beta_{1-42}/A\beta_{1-38}$ confirmed earlier findings [103, 104]. Although these previous studies pointed to a disease specific peptide pattern, our study shows that the added diagnostic value of such a pattern is questionable.

Regarding the pooled non-AD group the Aβ isoforms had no added diagnostic value, which is probably due to the fact this group is a combination of three pathophysiologically different disorders and the Aβ isoforms might behave differently in these different neurodegenerative disorders. Although the ratios of $A\beta_{1-42}$ increased the discriminative power of $A\beta_{1-42}$, analyzing Aβ isoforms did not have an added value for differentiating MCI or AD from pooled non-AD dementias as the routine biomarkers still performed better.

In summary, the diagnostic performance of $A\beta_{1-42}$ increased when calculating the $A\beta_{1-42}/A\beta_{1-40}$ ratio. This was the case when comparing the AD groups with FTD and when comparing MCI with non-AD in general, but also FTD, DLB and VaD separately and controls. Furthermore it was shown there is an added diagnostic value of the Aβ isoforms for differentiating AD and FTD. The added diagnostic value was only limited when comparing the AD groups with VaD, DLB and controls. Rather, altered $A\beta_{1-42}/A\beta_{1-40}$ ratios in CSF might be specific for AD since both peptides are representative for the two possible cleavage routes of the protease γ-secretase [114].

The present findings should be replicated and confirmed in a larger and independent cohort of patients, including autopsy-confirmed cases.

In conclusion, the Aβ isoforms could help in some differential diagnostic situations. Adding the Aβ isoforms to the current biomarker panel could enhance diagnostic accuracy. This is the case for discriminating AD from FTD and MCI from all other diagnoses and to diagnose AD in patients with normal $A\beta_{1\text{-}42}$ levels. In contrast to $A\beta_{1\text{-}42}$, Aβ isoforms seem to be correlated with disease severity in AD.

Table 3.1 Demographic, clinical and biomarker data for all groups.

	AD	**Non-AD**	**MCI**	**Controls**	**FTD**	**VaD**	**DLB**	***P* value**
Gender (F/M)	31 / 18	20 / 30	32 / 17	17 / 33	6 / 11	8 / 8	6 / 11	.002
Age (years)	77 (70-82)	74 (70-78)	79 (72-82)	68 (61-73)	70 (66-73)	77 (72-81)	75 (73-81)	<.001
% *APOE* ε4 carriers	56.8 *N; 44*	26.8 *N; 49*	40.0 *N; 40*	27.3 *N; 11*	29.4 *N; 17*	31.3 *N; 16*	25.0 *N; 16*	.04
MMSE at LP (/30)	19 (15-24) *N; 47*	18 (14-24) *N; 44*	25 (22-26) *N; 43*	Not available	19 (14-25) *N; 16*	16 (12-23) *N; 15*	19 (16-23) *N; 13*	<.001
Age at onset (years)	76 (68-80)	71 (65-77)	76 (67-80)	/	65 (59-68)	75 (69-79)	73 (69-78)	.04
Disease duration (years)	2 (1-4)	3 (1-4)	2 (1-4)	/	3 (2-6)	1 (1-3)	3 (2-3)	.97
$A\beta_{1-42}$ (pg/mL)	476 (389-578)	583 (417-853)	514 (406-616)	834 (630-1059)	582 (407-853)	603 (512-870)	595 (430-806)	<.001
tTau (pg/mL)	561 (405-807)	281 (230-428)	491 (422-603)	246 (172-373)	296 (230-428)	296 (230-431)	250 (234-377)	<.001
$pTau_{181}$ (pg/mL)	78.0 (58.0-98.0)	41.6 (32.0-59.8)	79.0 (63.0-105.0)	42.9 (33.0-61.7)	40.0 (32.0-61.0)	46.7 (37.8-58.4)	41.0 (30.0-49.0)	<.001
$A\beta_{1-37}$ (pg/mL)	701 (556-894)	544 (369-784)	943 (684-1135)	717 (530-885)	523 (384-706)	691 (471-905)	483 (369-758)	<.001
$A\beta_{1-38}$ (pg/mL)	2174 (1598-2880)	1679 (1108-2543)	2724 (2108-3872)	2238 (1745-2832)	1607 (1235-2212)	2070 (1340-2777)	1353 (1024-1968)	<.001
$A\beta_{1-40}$ (pg/mL)	6023 (4616-8447)	5120 (3757-7667)	8658 (7153-11310)	6380 (4599-8308)	4564 (3348-5799)	6680 (4045-7624)	4487 (3766-8005)	<.001
$A\beta_{1-42}$/tTau	.79 (.58-1.09)	2.18 (1.48-3.34)	.99 (.73-1.64)	3.30 (2.25-4.61)	2.06 (1.01-3.43)	2.27 (1.74-2.85)	2.41 (1.25-3.34)	<.001
$A\beta_{1-42}/pTau_{181}$	6.10 (4.67-7.16)	14.71 (8.78-20.75)	6.54 (4.61-9.14)	18.33 (13.40-23.24)	14.6 (7.05-20.90)	14.45 (9.72-21.07)	14.87 (8.72-19.66)	<.001

All data are median values with 25th and 75th quartiles between brackets, except for gender and % of *APOE* ε4 carriers. To compare gender distribution and *APOE* carrier status across the groups a Chi-square test was performed. A Kruskal-Wallis test was used to compare biomarker data over all groups.
Abbreviations: AD, Alzheimer's disease; Non-AD, dementia not due to Alzheimer's disease; MCI, mild cognitive impairment; FTD, frontotemporal dementia; VaD, vascular dementia; DLB, dementia with Lewy bodies; *APOE*, Apolipoprotein E; MMSE, Mini-Mental State Examination; Relative $A\beta_{1-42}$; ratio of $A\beta_{1-42}$ to the sum of all Aβ isoforms.

Table 3.1 (continued)

	AD	Non-AD	MCI	Controls	FTD	VaD	DLB	*P* value
$A\beta_{1-38}/A\beta_{1-40}$	.35 (.31-.38)	.33 (.28-.35)	.32 (.29-.35)	.35 (.32-.38)	.35 (.30-.37)	.34 (.31-.37)	.27 (.24-.33)	<.001
$A\beta_{1-42}/A\beta_{1-40}$	.07 (.06-.09)	.12 (.08-.17)	.06 (.04-.08)	.12 (.11-.16)	.14 (.09-.17)	.11 (.08-.15)	.13 (.08-.17)	<.001
$A\beta_{1-42}/A\beta_{1-38}$	.21 (.17-.26)	.40 (.24-.54)	.20 (.14-.26)	.37 (.29-.47)	.39 (.24-.48)	.31 (.25-.50)	.51 (.28-.60)	<.001
$A\beta_{1-42}/A\beta_{1-37}$	.66 (.51-.76)	1.13 (.79-1.57)	.62 (.41-.85)	1.19 (.97-1.48)	1.17 (.74-1.50)	.92 (.80-1.31)	1.22 (.83-1.72)	<.001
Relative $A\beta_{1-42}$	.05 (.04-.06)	.08 (.05-.11)	.04 (.03-.05)	.08 (.07-.10)	.09 (.06-.11)	.07 (.06-.10)	.09 (.05-.11)	<.001

All data are median values with 25th and 75th quartiles between brackets, except for gender and % of *APOE* ε4 carriers. To compare gender distribution and *APOE* carrier status across the groups a Chi-square test was performed. A Kruskal-Wallis test was used to compare biomarker data over all groups.
Abbreviations: AD, Alzheimer's disease; Non-AD, dementia not due to Alzheimer's disease; MCI, mild cognitive impairment; FTD, frontotemporal dementia; VaD, vascular dementia; DLB, dementia with Lewy bodies; *APOE*, Apolipoprotein E; MMSE, Mini-Mental State Examination; Relative $A\beta_{1-42}$; ratio of $A\beta_{1-42}$ to the sum of all Aβ isoforms.

Table 3.2 Correlation of the levels of the Aβ isoforms with MMSE scores, yearly MMSE score change and disease duration in the MCI and AD populations.

	$A\beta_{1-37}$	$A\beta_{1-38}$	$A\beta_{1-40}$	$A\beta_{1-42}$	$A\beta_{1-42}/A\beta_{1-40}$
Correlation with MMSE scores					
MCI population					
Correlation Coefficient	.207	.150	.248	.066	-.047
P-value	.182	.338	.109	.672	.765
N	43	43	43	43	43
AD population					
Correlation Coefficient	.520	.431	.450	.264	-.214
P value	.000	.003	.002	.073	.148
N	47	47	47	47	47
Correlation with yearly change in MMSE scores					
MCI population					
Correlation Coefficient	.239	.231	.099	,362	.187
P value	.148	.162	.554	.026	.261
N	38	38	38	38	38
AD population					
Correlation Coefficient	-.135	-.158	-.047	-.197	-.106
P value	.405	.330	.772	.223	.514
N	40	40	40	40	40
Correlation with disease duration					
MCI population					
Correlation Coefficient	-.063	.031	.013	.032	-.023
P value	.670	.832	.930	.827	.878
N	49	49	49	49	49
AD population					
Correlation Coefficient	.255	.370	.388	.034	-.397
P value	.077	.009	.006	.816	.005
N	49	49	49	49	49

Median change in MMSE over time in the AD group was -1.2 (-3.8-(-0.3)) over a median time interval of 2.7 years (1.3-4.5). In the MCI population the median MMSE change was -3.6 (-1.8-(-0.5)) over a median time interval of 3.6 years (2.4-5.8). Abbreviations: AD; Alzheimer's disease; MCI; mild cognitive impairment.

Table 3.3 Comparison of the Aβ isoforms between *APOE* ε4 carriers and non-carriers.

	N	$A\beta_{1-37}$ (pg/mL)	$A\beta_{1-38}$ (pg/mL)	$A\beta_{1-40}$ (pg/mL)	$A\beta_{1-42}$ (pg/mL)
All groups					
Non-carrier	86	691 (497-894)	2111 (1456-2804)	6669 (4426-8585)	578 (433-824)
Carrier	58	707 (556-943)	2186 (1607-2831)	6251 (5018-8664)	469 (378-548)
***P* value**		.273	.489	.824	.000
AD population					
Non-carrier	19	593 (518-882)	1934 (1488-2575)	5497 (4329-7230)	500 (417-600)
Carrier	25	701 (591-888)	2018 (1797-2631)	5928 (5018-7580)	443 (321-508)
***P* value**		.118	.678	.337	.110
MCI population					
Non-carrier	24	865 (648-1081)	2661 (2069-3410)	8650 (7004-11292)	520 (421-621)
Carrier	16	951 (698-1119)	2756 (2268-3605)	8553 (7671-10104)	495 (330-577)
***P* value**		.629	.679	.679	.263
Combination MCI and AD					
Non-carrier	43	742 (536-980)	2427 (1644-3267)	7230 (5326-9535)	513 (417-606)
Carrier	41	727 (637-971)	2300 (1856-2880)	6843 (5403-8956)	462 (321-541)
***P* value**		.579	.961	.690	.040
Non-AD population					
Non-carrier	35	562 (414-768)	1679 (1108-2576)	5388 (3821-7667)	714 (509-915)
Carrier	14	558 (369-866)	1715 (1225-2543)	5120 (3348-8005)	502 (397-584)
***P* value**		.982	.965	.912	.026

All data are median values with 25th and 75th quartiles between brackets, except for N.
Abbreviations: AD; Alzheimer's disease; Non-AD; dementia not due to Alzheimer's disease; MCI; mild cognitive impairment.

Table 3.4 Best performing biomarkers for all differential diagnostic situations based on ROC curve analyses.

	AD versus controls					**MCI versus controls**			
	AUC	cut-off	sens [%]	spec [%]		AUC	cut-off	sens [%]	spec [%]
$A\beta_{1-42}$/tTau	0.968	< 1.708	93.9	92.0	$A\beta_{1-42}$/tTau	0.922	< 1.861	83.7	90.0
$A\beta_{1-42}$/$pTau_{181}$	0.930	< 10.122	91.8	86.0	$A\beta_{1-42}$/$A\beta_{1-40}$	0.924	< 0.1024	91.8	84.0
	AD versus non-AD					**MCI versus non-AD**			
$A\beta_{1-42}$/tTau	0.842	< 1.420	87.8	76.0	$pTau_{181}$	0.857	> 57.50 pg/mL	89.8	74.0
$A\beta_{1-42}$/$pTau_{181}$	0.840	< 9.440	87.8	72.0	$A\beta_{1-42}$/$A\beta_{1-40}$	0.845	< 0.1022	91.8	62.0
	AD versus FTD					**MCI versus FTD**			
Relative $A\beta_{1-42}$	0.831	< 0.0768	91.8	58.8	Relative $A\beta_{1-42}$	0.875	< 0.0491	67.3	94.1
$A\beta_{1-42}$/$A\beta_{1-37}$	0.851	< 0.7351	69.4	94.1	$A\beta_{1-42}$/$A\beta_{1-40}$	0.882	< 0.0944	85.7	75.0
	AD versus VaD					**MCI versus VaD**			
$A\beta_{1-42}$/tTau	0.902	< 1.589	89.8	87.5	$pTau_{181}$	0.881	> 59.90 pg/mL	85.7	81.3
$A\beta_{1-42}$/$pTau_{181}$	0.912	< 8.096	79.6	93.8	$A\beta_{1-42}$/$pTau_{181}$	0.860	< 8.092	67.3	93.8
	AD versus DLB					**MCI versus DLB**			
$A\beta_{1-42}$/$A\beta_{1-38}$	0.843	< 0.3957	95.9	70.6	$pTau_{181}$	0.855	> 49.50 pg/mL	95.9	76.5
$A\beta_{1-42}$/tTau	0.838	< 1.222	83.7	76.5	$A\beta_{1-38}$	0.855	> 1850.00 pg/mL	87.8	70.6

Abbreviations: AD; Alzheimer's disease; AUC; area under the curve; MCI; mild cognitive impairment; Relative $A\beta_{1-42}$; ratio of $A\beta_{1-42}$ to the sum of all Aβ isoforms; sens; sensitivity; spec; specificity.

Table 3.5 Significance levels (*P* values) of the AUC value comparisons of the Aβ isoforms ratios with $A\beta_{1\text{-}42}$ alone.

	$A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$	**$A\beta_{1\text{-}42}/A\beta_{1\text{-}38}$**	**$A\beta_{1\text{-}42}/A\beta_{1\text{-}37}$**
AD versus controls	.857	.688	.918
MCI versus controls	.002	.102	.049
AD versus non-AD	.113	.049	.016
MCI versus non-AD	<.001	<.001	<.001
AD versus FTD	.025	.039	.008
MCI versus FTD	<.001	.002	.001
AD versus VaD	.979	.899	.899
MCI versus VaD	.034	.133	.103
AD versus DLB	.392	.058	.061
MCI versus DLB	.009	.002	.002

DeLong tests were performed by using the pROC package in the statistical software package R to compare the AUC values. Abbreviations: AUC; area under the curve; AD; Alzheimer's disease; Non-AD; dementia not due to Alzheimer's disease; MCI; mild cognitive impairment; FTD; frontotemporal dementia; VaD; vascular dementia; DLB; dementia with Lewy bodies.

Supplementary material

Supplementary table 3.1 ROC curve analyses comparing AD and MCI.

	AUC	cut-off	sens [%]	spec [%]
$A\beta_{1-42}$	0.575	< 527.06 pg/mL	71.4	46.9
tTau	0.579	> 536.50 pg/mL	57.1	65.3
$pTau_{181}$	0.449	> 86.85 pg/mL	40.8	63.3
$A\beta_{1-42}$/tTau	0.600	< 1.314	85.7	34.7
$A\beta_{1-42}/pTau_{181}$	0.539	< 7.278	77.6	44.9
$A\beta_{1-37}$	0.680	< 908.00 pg/mL	77.6	55.1
$A\beta_{1-38}$	0.671	< 2422.50 pg/mL	65.3	67.3
$A\beta_{1-40}$	0.734	< 6866.00 pg/mL	63.3	81.6
$A\beta_{1-38}/A\beta_{1-40}$	0.684	> 0.368	46.9	89.8
$A\beta_{1-42}/A\beta_{1-40}$	0.626	> 0.0574	75.5	49.0
$A\beta_{1-42}/A\beta_{1-38}$	0.566	> 0.1573	85.7	36.7
$A\beta_{1-42}/A\beta_{1-37}$	0.570	> 0.4195	89.9	30.6
Relative $A\beta_{1-42}$	0.607	> 0.0370	81.6	42.9

Abbreviations: AUC; area under the curve; Relative $A\beta_{1-42}$; ratio of $A\beta_{1-42}$ to the sum of all Aβ isoforms; sens; sensitivity; spec; specificity.

Supplementary table 3.2 ROC curve analyses comparing AD and controls.

	AD versus controls				MCI versus controls			
	AUC	**cut-off**	**sens [%]**	**spec [%]**	**AUC**	**cut-off**	**sens [%]**	**spec [%]**
$A\beta_{1-42}$	0.874	< 722.00 pg/mL	98.0	74.0	0.793	< 712.00 pg/mL	85.7	74.0
tTau	0.889	> 333.00 pg/mL	87.8	72.0	0.877	> 319.27 pg/mL	93.9	70.0
$pTau_{181}$	0.804	> 51.60 pg/mL	87.8	62.0	0.844	> 54.85 pg/mL	93.9	64.0
$A\beta_{1-42}$/tTau	0.968	< 1.708	93.9	92.0	0.922	< 1.861	83.7	90.0
$A\beta_{1-42}/pTau_{181}$	0.930	< 10.122	91.8	86.0	0.905	< 11.110	87.8	82.0
$A\beta_{1-37}$	0.522	> 501.00 pg/mL	89.8	20.0	0.694	> 901.00 pg/mL	55.1	78.0
$A\beta_{1-38}$	0.482	> 1332.00 pg/mL	93.9	14.0	0.650	> 2321.50 pg/mL	69.4	58.0
$A\beta_{1-40}$	0.499	> 3425.50 pg/mL	100.0	10.0	0.755	> 7909.50 pg/mL	69.4	74.0
$A\beta_{1-38}/A\beta_{1-40}$	0.464	> 0.346	59.2	50.0	0.708	< 0.328	65.3	72.0
$A\beta_{1-42}/A\beta_{1-40}$	0.881	< 0.1099	85.7	78.0	0.924	< 0.1024	91.8	84.0
$A\beta_{1-42}/A\beta_{1-38}$	0.858	< 0.2690	81.6	82.0	0.867	< 0.2850	83.7	80.0
$A\beta_{1-42}/A\beta_{1-37}$	0.870	< 0.7861	77.6	90.0	0.877	< 0.9353	83.7	80.0
Relative $A\beta_{1-42}$	0.878	< 0.0593	75.5	90.0	0.913	< 0.0636	87.8	84.0

Abbreviations: AUC; area under the curve; Relative $A\beta_{1-42}$; ratio of $A\beta_{1-42}$ to the sum of all Aβ isoforms; sens; sensitivity; spec; specificity.

Supplementary table 3.3 ROC curve analyses comparing AD and non-AD.

	AD versus non-AD				MCI versus non-AD			
	AUC	**cut-off**	**sens [%]**	**spec [%]**	**AUC**	**cut-off**	**sens [%]**	**spec [%]**
$A\beta_{1-42}$	0.686	< 694.00 pg/mL	95.9	40.0	0.606	< 706.50 pg/mL	85.7	40.0
tTau	0.819	> 335.50 pg/mL	87.8	64.0	0.799	> 317.34 pg/mL	93.9	62.0
$pTau_{181}$	0.820	> 50.00 pg/mL	91.8	66.0	0.857	> 57.50 pg/mL	89.8	74.0
$A\beta_{1-42}$/tTau	0.842	< 1.420	87.8	76.0	0.765	< 1.563	73.5	74.0
$A\beta_{1-42}/pTau_{181}$	0.840	< 9.440	87.8	72.0	0.803	< 12.855	89.8	62.0
$A\beta_{1-37}$	0.659	> 501.50 pg/mL	89.8	44.0	0.785	> 941.50 pg/mL	51.0	92.0
$A\beta_{1-38}$	0.657	> 1396.50 pg/mL	91.8	40.0	0.778	> 1850.00 pg/mL	87.8	60.0
$A\beta_{1-40}$	0.615	> 3849.00 pg/mL	95.9	30.0	0.802	> 5849.00 pg/mL	89.8	62.0
$A\beta_{1-38}/A\beta_{1-40}$	0.637	> 0.354	53.1	76.0	0.532	< 0.330	65.3	50.0
$A\beta_{1-42}/A\beta_{1-40}$	0.782	< 0.1215	93.9	50.0	0.845	< 0.1022	91.8	62.0
$A\beta_{1-42}/A\beta_{1-38}$	0.804	< 0.2730	81.6	68.0	0.822	< 0.3281	89.8	58.0
$A\beta_{1-42}/A\beta_{1-37}$	0.821	< 0.7351	69.4	86.0	0.822	< 0.6871	65.3	88.0
Relative $A\beta_{1-42}$	0.791	< 0.0737	89.8	56.0	0.843	< 0.0635	87.8	66.0

Abbreviations: AUC; area under the curve; Relative $A\beta_{1-42}$; ratio of $A\beta_{1-42}$ to the sum of all Aβ isoforms; sens; sensitivity; spec; specificity.

Supplementary table 3.4 ROC curve analyses comparing AD and FTD.

	AD versus FTD				MCI versus FTD			
	AUC	**cut-off**	**sens [%]**	**spec [%]**	**AUC**	**cut-off**	**sens [%]**	**spec [%]**
$A\beta_{1-42}$	0.625	< 751.00 pg/mL	98.0	35.3	0.562	< 770.00 pg/mL	89.8	35.3
tTau	0.775	> 435.17 pg/mL	71.4	76.5	0.756	> 301.98 pg/mL	95.9	52.9
$pTau_{181}$	0.799	> 48.50 pg/mL	93.9	64.7	0.837	> 56.50 pg/mL	91.8	70.6
$A\beta_{1-42}$/tTau	0.791	< 1.420	87.8	70.6	0.718	< 1.871	83.7	58.8
$A\beta_{1-42}/pTau_{181}$	0.815	< 9.629	89.8	70.6	0.780	< 9.458	79.6	70.6
$A\beta_{1-37}$	0.720	> 530.00 pg/mL	81.6	64.7	0.812	> 526.00 pg/mL	91.8	64.7
$A\beta_{1-38}$	0.685	> 1745.50 pg/mL	69.4	70.6	0.795	> 1702.00 pg/mL	89.8	70.6
$A\beta_{1-40}$	0.687	> 4050.50 pg/mL	91.8	41.2	0.842	> 5849.00 g/mL	89.8	76.5
$A\beta_{1-38}/A\beta_{1-40}$	0.541	> 0.353	53.1	70.6	0.647	< 0.331	65.3	70.6
$A\beta_{1-42}/A\beta_{1-40}$	0.830	< 0.0939	75.5	75.0	0.882	< 0.0944	85.7	75.0
$A\beta_{1-42}/A\beta_{1-38}$	0.815	< 0.2754	81.6	68.8	0.825	< 0.2600	75.5	75.0
$A\beta_{1-42}/A\beta_{1-37}$	0.851	< 0.7351	69.4	94.1	0.842	< 0.7334	67.3	94.1
Relative $A\beta_{1-42}$	0.831	< 0.0768	91.8	58.8	0.875	< 0.0491	67.3	94.1

Abbreviations: AUC; area under the curve; Relative $A\beta_{1-42}$; ratio of $A\beta_{1-42}$ to the sum of all Aβ isoforms; sens; sensitivity; spec; specificity.

Supplementary table 3.5 ROC curve analyses comparing AD and VaD.

	AD versus VaD				MCI versus VaD			
	AUC	**cut-off**	**sens [%]**	**spec [%]**	**AUC**	**cut-off**	**sens [%]**	**spec [%]**
$A\beta_{1-42}$	0.766	< 508.50 pg/mL	63.3	81.3	0.663	< 709.00 pg/mL	85.7	43.8
tTau	0.849	> 313.72 pg/mL	89.8	68.8	0.828	> 317.34 pg/mL	93.9	68.8
$pTau_{181}$	0.833	> 60.40 pg/mL	71.4	81.3	0.881	> 59.90 pg/mL	85.7	81.3
$A\beta_{1-42}$/tTau	0.902	< 1.589	89.8	87.5	0.818	< 1.569	73.5	87.5
$A\beta_{1-42}/pTau_{181}$	0.912	< 8.096	79.6	93.8	0.860	< 8.092	67.3	93.8
$A\beta_{1-37}$	0.526	> 369.00 pg/mL	100.0	25.0	0.695	> 941.50 pg/mL	51.0	87.5
$A\beta_{1-38}$	0.533	> 1046.50 pg/mL	100.0	25.0	0.679	> 2397.00 pg/mL	67.3	62.5
$A\beta_{1-40}$	0.524	> 3227.00 pg/mL	100.0	25.0	0.753	> 7801.50 pg/mL	69.4	81.3
$A\beta_{1-38}/A\beta_{1-40}$	0.538	> 0.317	73.5	43.8	0.643	< 0.352	79.6	50.0
$A\beta_{1-42}/A\beta_{1-40}$	0.763	< 0.0939	75.5	75.0	0.846	< 0.0944	85.7	75.0
$A\beta_{1-42}/A\beta_{1-38}$	0.753	< 0.2754	81.6	68.8	0.793	< 0.2600	75.5	75.0
$A\beta_{1-42}/A\beta_{1-37}$	0.777	< 0.7871	77.6	81.3	0.791	< 0.7942	73.5	81.3
Relative $A\beta_{1-42}$	0.758	< 0.0608	75.5	75.0	0.829	< 0.0611	83.7	75.0

Abbreviations: AUC; area under the curve; Relative $A\beta_{1-42}$; ratio of $A\beta_{1-42}$ to the sum of all Aβ isoforms; sens; sensitivity; spec; specificity.

Supplementary table 3.6 ROC curve analyses comparing AD and DLB.

	AD versus DLB				MCI versus DLB			
	AUC	**cut-off**	**sens [%]**	**spec [%]**	**AUC**	**cut-off**	**sens [%]**	**spec [%]**
$A\beta_{1-42}$	0.672	< 534.00 pg/mL	71.4	64.7	0.596	< 706.50 pg/mL	85.7	41.2
tTau	0.836	> 282.50 pg/mL	95.5	64.7	0.815	> 284.50 pg/mL	98.0	64.7
$pTau_{181}$	0.830	> 50.00 pg/mL	91.8	76.5	0.855	> 49.50 pg/mL	95.9	76.5
$A\beta_{1-42}$/tTau	0.838	< 1.222	83.7	76.5	0.762	< 2.285	87.8	58.8
$A\beta_{1-42}/pTau_{181}$	0.798	< 14.074	95.9	64.7	0.773	< 13.463	89.8	64.7
$A\beta_{1-37}$	0.725	> 495.00 pg/mL	89.8	58.8	0.844	< 486.50 pg/mL	95.9	58.8
$A\beta_{1-38}$	0.745	> 1378.00 pg/mL	91.8	52.9	0.855	> 1850.00 pg/mL	87.8	70.6
$A\beta_{1-40}$	0.630	> 4492.50 pg/mL	79.6	52.9	0.810	> 5760.00 pg/mL	89.8	64.7
$A\beta_{1-38}/A\beta_{1-40}$	0.826	> 0.342	61.2	94.1	0.688	> 0.274	85.7	52.9
$A\beta_{1-42}/A\beta_{1-40}$	0.753	< 0.1162	89.8	58.8	0.807	< 0.1029	91.8	64.7
$A\beta_{1-42}/A\beta_{1-38}$	0.843	< 0.3957	95.9	70.6	0.845	< 0.4661	98.0	64.7
$A\beta_{1-42}/A\beta_{1-37}$	0.832	< 1.0406	89.8	70.6	0.831	< 1.0325	87.8	70.6
Relative $A\beta_{1-42}$	0.783	< 0.0816	95.9	58.8	0.825	< 0.0637	87.8	70.6

Abbreviations: AUC; area under the curve; Relative $A\beta_{1-42}$; ratio of $A\beta_{1-42}$ to the sum of all Aβ isoforms; sens; sensitivity; spec; specificity.

3.2 No value for cerebrospinal fluid β-site amyloid precursor protein cleavage enzyme 1 (BACE1) levels in predicting cognitive decline in mild cognitive impairment

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Manuscript in preparation.

Contribution: Selection of patients, collection of patient data and samples, data quality control, statistical analyses, interpretation of results, literature review, writing of paper

Abstract

As $A\beta_{1-42}$ is currently regarded as the earliest biomarker that changes in AD, additional markers related to Aβ metabolism and pathology may be of great value to increase the accuracy of early-stage diagnosis and prognosis, but also to measure target engagement of candidate drugs in clinical trials targeting this specific pathophysiological process. As such, an interesting biomarker candidate would be the presynaptic β-site amyloid precursor protein cleavage enzyme 1 (BACE1, also shortened to β-secretase), one of the key enzymes involved in Aβ production.

Here, we investigated whether increase in BACE1 in CSF may be an early indicator of forthcoming AD or may be predictive of cognitive decline in a heterogeneous MCI population and/or in a homogeneous probable AD dementia population with biomarker-based diagnosis.

The study population consisted of 83 MCI patients with clinical follow-up, 27 probable AD dementia patients with biomarker evidence of AD pathology, and 20 cognitively healthy individuals. Of the 83 MCI patients, 49 progressed to probable AD dementia during follow-up (MCI-AD), 6 progressed to a dementia syndrome other than AD (MCI-nonAD), while 28 MCI patients stayed stable during a follow-up period of at least one year (MCI stable).

Logistic and linear regression models evaluated clinical progression to AD dementia and MMSE decline, respectively, as a function of baseline biomarker levels. Subsequent cross-validation determined the power to predict progression and MMSE decline.

No differences in BACE1 CSF levels were found between the baseline groups, nor between the follow-up groups. In addition, BACE1 levels were not predictive of progression to dementia or MMSE decline.

In conclusion, even though BACE1 holds theoretical promise as a biomarker for AD pathology, measuring BACE1 levels in CSF has not proven to be useful for clinical purposes.

Introduction

Though the combination of the core AD biomarkers are highly accurate for AD, only $A\beta_{1-42}$ is regarded as a really early marker [115] and thus new additional biomarkers would be of great value for early-stage diagnosis but also to measure target engagement of candidate drugs in clinical trials targeting early pathological changes. As such, one of the important pathways in AD is the processing in which amyloid precursor protein (APP) is first cleaved by β-site amyloid precursor protein cleavage enzyme 1 (BACE1, also shortened to β-secretase), followed by γ-secretase cleavage [116, 117]. Increased activity and protein levels of BACE1 have been reported in the brain of sporadic AD patients [118-122]. Moreover, previous studies also found increased BACE1 activity in CSF of AD compared with controls [123] but also with non-AD dementia patients [124]. An increased BACE1 activity has also been found in subjects with a positive AD biomarker profile (decreased $A\beta_{1-42}$ and increased tTau and/or $pTau_{181}$) [125] as well as to be associated with a decreased hippocampal volume in patients with AD [126]. In addition, increased BACE1 activity has been found in MCI subjects that progress to AD at follow-up [127]. Furthermore, one study has indicated BACE1 importance in early detection as both its concentration and activity was increased in MCI patients compared with both controls and AD patients [128]. The findings on BACE1 protein levels in CSF, however, are contradictory, with some studies showing (slightly) increased CSF levels in MCI and AD compared with healthy subjects [128], while others show no significantly altered levels [129, 130]. It has also been shown that BACE1 levels in CSF mainly correlate with tTau and $pTau_{181}$ levels in the CSF, suggesting that alterations in BACE1 are associated with cell death and neurodegeneration rather than early pathology [131]. These conflicting results clearly need further clarification.

Therefore, in this study we investigated whether BACE1 protein levels are increased in a heterogeneous MCI population and/or in a homogeneous probable AD dementia population with biomarker-based diagnosis, and whether these changes have a value to predict cognitive decline in MCI. This study was designed as a pilot study to identify biomarkers that should be considered for a prospective validation study.

Materials and methods

Study population

Samples from patients and controls were retrospectively selected from the Biobank of the Institute Born-Bunge. Only samples from patients recruited in the Memory Clinic and Department of Neurology of Hospital Network Antwerp (ZNA) Middelheim and Hoge Beuken were selected to avoid inter-center variability due to possible differences in pre-analytical steps.

The study population consisted of three groups. The first included 83 MCI patients with clinical follow-up. Of the 83 MCI patients, 49 progressed to probable AD dementia during follow-up (MCI-AD), 6 progressed to a dementia syndrome other than AD (MCI-nonAD), while 28 MCI patients stayed stable during a follow-up period of at least one year (MCI stable). A diagnosis of MCI and progression to probable AD dementia was established based on the clinical criteria of Petersen [71] and NINCDS/ADRDA [4], respectively. The diagnostic work-up included a general physical and neurological examination, routine blood examination, structural brain imaging, Mini-Mental State Examination (MMSE), and extensive neuropsychological examination adjusted for age and education.

For cross-sectional comparison purposes, the population also included 27 probable AD dementia patients and 20 cognitively healthy controls (CO). The patients with probable AD dementia at baseline all complied with the biomarker-based NIA-AA research criteria [5] and underwent the same diagnostic work-up as the MCI patients. All CO subjects underwent a full neuropsychological assessment to rule out cognitive decline and met the following criteria: (1) no neurological or psychiatric history, (2) no organic disease involving the central nervous system, and (3) no abnormal CSF biomarkers indicating underlying AD pathology.

The study was approved by the local ethics committee and all subjects gave written informed consent.

Cognitive decline in MCI subjects

Cognitive decline was first assessed by progression to AD dementia during clinical follow-up. Second, cognitive decline was evaluated by decline in MMSE score during clinical follow-up. In order to calculate MMSE decline, a linear regression was fitted through each individuals MMSE scores over time starting from LP. The slope of the regression line was used as a measure of decline. Next, all analyses were weighted for the goodness-of-fit of the individual regression line as well as for the number of MMSE scores. The better the fit and/or the more MMSE scores, the higher the individuals weight in the analyses. One MCI subject lacked follow-up MMSE scores and was thus excluded from these analyses.

CSF sampling

LP, CSF sampling and handling have been performed according to a standard protocol [53]. CSF samples were stored at -80°C until analysis.

CSF analyses

The core AD CSF biomarkers $A\beta_{1-42}$, $pTau_{181}$, and tTau were measured with the corresponding INNOTEST® assays (Fujirebio Europe, INNOTEST® β-$AMYLOID_{(1-42)}$, $PHOSPHO\text{-}TAU_{(181P)}$, and hTau Ag, respectively). To analyze BACE1 the BACE-1 ELISA EQ 6541-9601-L of EUROIMMUN was used. The intra- and inter-assay CVs were below 13%. The samples were randomized and the analyses were done blinded for diagnosis.

Biomarker-based stratification of participants

For analyses purposes, the participants were stratified into two biomarker groups according to their A (amyloid) and T (neurofibrillary tangles) status (AT status) [34], based on in-house validated thresholds (in autopsy-confirmed AD versus cognitively healthy elderly) [112, 132] of CSF $A\beta_{1-42}$ (<638.5 pg/mL) and $pTau_{181}$ (>56.5 pg/mL), respectively. The subjects were stratified as being abnormal on both A and T (A+T+) or not.

Statistical analyses

All analyses were performed using the integrated development environment for R programming language, RStudio (version 1.0.136) [133]. First, non-parametric tests were used to test for significant differences of demographic characteristics and biomarker levels between groups. Differences in categorical variables were assessed by a χ^2 test. Bonferroni was used to correct *P* values for multiple comparisons, and a significance level of .05 was used to interpret the results. Post hoc analysis provided significant differences between groups.

Next, prediction of progression to dementia was assessed by logistic regression analyses in a subpopulation including only MCI stable and MCI-AD, using progression as outcome and biomarker levels as main effect, corrected for age and gender.

Prediction of MMSE decline was evaluated by linear regression analyses in the entire MCI population (MCI stable, MCI-AD, and MCI-nonAD), using MMSE decline as outcome and biomarker levels as main effect, corrected for age, gender, and baseline MMSE.

Both analyses above were also performed with AT status, with biomarker group as main effect and correcting for the same covariates.

In order to compare the predictive powers of the biomarkers and AT status, 100x 10-fold cross-validation was performed, using R package 'caret' [134]. The predictive power of logistic regression was assessed by prediction accuracy, while that of linear regression was evaluated by the root-mean-square error (RMSE), normalized to the units of each marker to enable comparison between biomarkers.

Results

Demographic and biomarker characteristics are summarized in Table 3.6. The CO group was significantly younger than the MCI and AD dementia groups. Demographic and biomarker characteristics of the MCI population are presented in Table 3.7. The MCI stable group was significantly younger than the MCI-AD group.

Comparison between baseline and follow-up diagnoses

Figure 3.1 summarizes the biomarker levels of the CO, MCI, and AD groups. The levels of all core AD biomarkers differed significantly across baseline diagnoses. The levels of BACE1, however, did not differ significantly across the groups.

The biomarker levels of the MCI stable, MCI-AD, and MCI-nonAD groups are shown in Figure 3.2. The levels of BACE1 across the groups, being specifically lower in MCI-nonAD compared with MCI-AD.

In addition, we stratified the patients according to their biomarker profile, as defined by the IWG-2 criteria [33] (i.e. decreased CSF $A\beta_{1\text{-}42}$ together with increased $pTau_{181}$ and/or tTau). As shown in Figure 3.3, we did not find any differences between those with an AD biomarker profile (IWG2 positive) and those without (IWG2 negative).

Prediction of progression to AD dementia

Logistic regression analyses revealed that A+T+ status (*P*=.0097) and high levels of $pTau_{181}$ (*P*=.0113), and tTau (*P*=.0096) predict progression from MCI to AD dementia. Consequent cross-validation produced highest accuracy levels for AT status (Figure 3.4).

Neither in the A+T+ nor in non-A+T+ MCI subgroups did any of the biomarkers significantly predict progression to AD dementia.

Prediction of MMSE decline

Linear regression analyses indicated that low $A\beta_{1\text{-}42}$ (*P*=.0340), and high $pTau_{181}$ (*P*=.0489), and tTau (*P*=.0007) predict MMSE decline in MCI. Cross-validation consequently showed that tTau has the lowest error to predict MMSE decline (Figure 3.5).

In the A+T+ MCI subjects, only high $pTau_{181}$ predicted MMSE decline (*P*=.0460; normalized RMSE shown in Figure 3.5), while high $pTau_{181}$ (*P*=.0003), and tTau (*P*=.0005) levels predicted MMSE decline in MCI subjects who were not A+T+. In this

latter group, $pTau_{181}$ rendered the lowest prediction error based on cross-validation (Figure 3.5). BACE1 did not predict MMSE decline in any of the groups.

Discussion

The aim of this pilot study was to assess whether BACE1 levels are increased in a heterogeneous MCI population and/or in a homogeneous probable AD dementia population with biomarker-based diagnosis, and second, whether BACE1 has a value to predict cognitive decline in MCI.

First, when comparing baseline diagnoses and follow-up diagnoses, we found no differences between baseline diagnostic groups. Interestingly, the levels of BACE1 were higher in subjects progressing to probable AD dementia compared with those progressing to another dementia disorder. This could indicate at least that BACE1 levels are related to AD. Yet, we did not find a difference between subjects with and without an AD biomarker profile as established by the IWG-2 criteria [33], which contradicts a possible specificity of BACE1 levels for AD.

Second, no predictive power of BACE1 was detected in this study, neither to predict progression to AD dementia nor to predict MMSE decline. Given there were no significant or striking differences in BACE1 levels between baseline and follow-up diagnostic groups, this finding is not surprising.

Although having theoretical potential as a biomarker, BACE1 protein levels in CSF have so far failed to fulfil expectations. This could possibly be due to the fact that the assay for BACE1 measures total BACE1 levels. During transport from the endoplasmic reticulum to the cell surface, BACE1 undergoes post-translational modifications. One of which, complex *N*-glycosylation, is an important process in the maturation of BACE1, leading to a substantial increase in molecular mass, changes in protein folding as well as increased stability [135, 136]. Maturation of BACE1 contributes to its enzymatic activity and thus to Aβ production. As both mature and immature forms of BACE1 protein exist in the CSF [128], total BACE1 levels include both forms. As such, it would be interesting to investigate whether an assay for mature BACE1 produces better results than a less specific total BACE1 assay.

In addition, given that BACE1 is an intracellular, transmembrane presynaptic protein, one could even argue whether increased CSF levels are due to increased activity or rather due to synaptic and/or neuronal degeneration. This hypothesis is also supported by its high colinearity with levels of $pTau_{181}$ and tTau. A ligand targeting BACE1 on positron emission tomography (PET) might be more successful in detecting changes in BACE1 in AD or assessing target engagement of a BACE inhibitor than total BACE1 levels in CSF [137].

While this pilot study benefits from strengths like a clinically well-characterized and representative population as well as medium-to-long follow-up of the MCI subjects, it also has several limitations. For one, the relatively small number of MCI subjects did not enable us to investigate the predictive powers of the biomarkers in more specific subgroups, such as A-T-, A-T+, and A+T- separately. However, as it is unclear what pathology is driving the cognitive impairment in these particular subgroups, we found it reasonable to combine these three categories into a heterogeneous non-A+T+ MCI group. Secondly, the large majority of MCI subjects progressed to AD dementia, which corresponds to the population visiting our memory clinic. The very small number of MCI subjects progressing to another disease than AD, however, did not allow us to draw any conclusions regarding progression to MCI-nonAD. Thirdly, as we retrospectively selected our study cohort, various tests were used in the neuropsychological examinations that were part of the diagnostic work-up, depending on the diagnostic question and of the abilities of the patient. As a result, not all patients underwent the same tests. Only MMSE was common in the neuropsychological test battery of all patients, which limited the measure of cognitive decline to MMSE decline only. It would therefore be interesting to repeat this study using the same full neuropsychological assessment for the entire population. Finally, due to co-linearity of the biomarkers, it was not appropriate to test multi-biomarker models, to assess whether BACE1 improved the performance of the core AD biomarkers alone. As such, we were only able to test single-biomarker models.

In conclusion, BACE1 holds theoretical promise as a biomarker for AD diagnosis or to measure target engagement of BACE1-targeting drugs in clinical trials. Yet, measuring total BACE1 levels in CSF has so far, including in this study, produced

disappointing results. Measuring only mature BACE1 levels in CSF might improve diagnostic performance, as well as measuring BACE1 on PET.

Table 3.6 Demographic and biomarker characteristics of the baseline population.

		CO	MCI	AD	*P* values
N (%F)		20 (60.0%)	83 (57.8%)	27 (51.9%)	.824
Age at LP, years		62.5 (60.6-64.6)[a,b]	75.0 (70.2-80.0)	74.9 (68.4-79.4)	<.0001
MMSE at LP		30 (28.8-30)[a,b]	25 (23-27)[a]	21 (18-27)	<.0001
AT status, N (%)	**A-T-**	20 (100%)[a,b]	20 (24%)	0 (0%)	<0.001
	A-T+	0 (0%)	11 (13%)	0 (0%)	
	A+T-	0 (0%)	19 (23%)	6 (22%)	
	A+T+	0 (0%)	33 (40%)	21 (78%)	

Note: Data are presented as median (25th-75th percentiles), except for N and AT status. *P* values indicate the values assessed with Kruskal-Wallis for each variable except gender and AT status, where a χ^2 was performed. Post hoc analysis provided significant differences between groups: [a]from AD, [b]from MCI. Abbreviations: AD, Alzheimer's disease; AT status, amyloid and tau status; CO, cognitively healthy; LP, lumbar puncture; MCI, mild cognitive impairment; MMSE, mini-mental state examination

Table 3.7 Demographic and biomarker characteristics of the MCI population.

		MCI stable	MCI-AD	MCI-nonAD	*P* value
N (%F)		28 (53.6%)	49 (61.2%)	6 (50%)	.914
Age at LP, years		72.5 (69.0-76.0)[a]	77.5 (72.2-81.0)	74.5 (72.5-75.0)	.031
Follow-up time, years*		3.19 (1.79-4.70)	2.23 (1.20-3.44)	3.52 (2.94-5.19)	.051°
MMSE at LP		27 (25-28)[a]	25 (22-27)	25 (23-27)	.008
A/T status, N (%)	**A-T-**	7 (25%)	9 (19%)	4 (67%)	.004
	A-T+	4 (14%)	7 (14%)	0 (0%)	
	A+T-	10 (36%)	7 (14%)	2 (33%)	
	A+T+	7 (25%)	26 (53%)	0 (0%)	

Note: Data are presented as median (25th-75th percentiles), except for N and AT status. *P* values indicate the values assessed with Kruskal-Wallis for each variable except gender and AT status, where a χ^2 was performed. Post hoc analysis provided significant differences between groups: [a]from MCI-AD, [b]from MCI-nonAD. Abbreviations: AD, Alzheimer's disease; AT status, amyloid and tau status; CO, cognitively healthy; LP, lumbar puncture; MCI, mild cognitive impairment; MCI-AD, MCI progressing to AD dementia; MCI-nonAD, MCI progressing to dementia other than AD; MMSE, mini-mental state examination

*Time from LP to last consultation (MCI stable) or progression to dementia.

°*P* value of difference between MCI-AD and MCI-nonAD

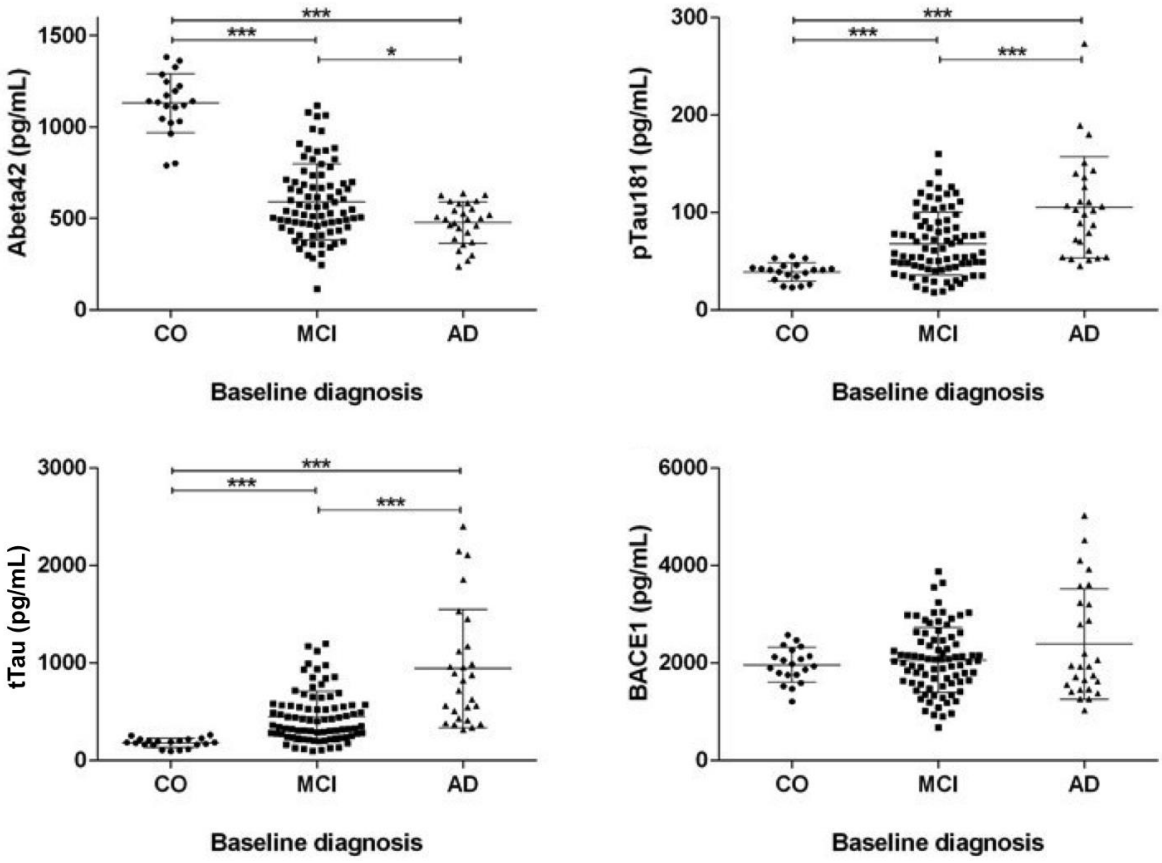

Figure 3.1 Biomarker levels according to baseline diagnosis. *P* value indicators correspond to the values assessed with Mann-Whitney U. Post hoc analysis provided significant differences between groups: * *P*<.0167; ** *P*<.005; *** *P*<.001. Abbreviations: Abeta42, amyloid-β protein of 42 amino-acids; AD, probable Alzheimer's disease dementia; BACE1, β-site amyloid precursor protein cleavage enzyme 1; CO, cognitively healthy; MCI, mild cognitive impairment, pTau181, tau protein phosphorylated at threonine 181; tTau, total tau protein.

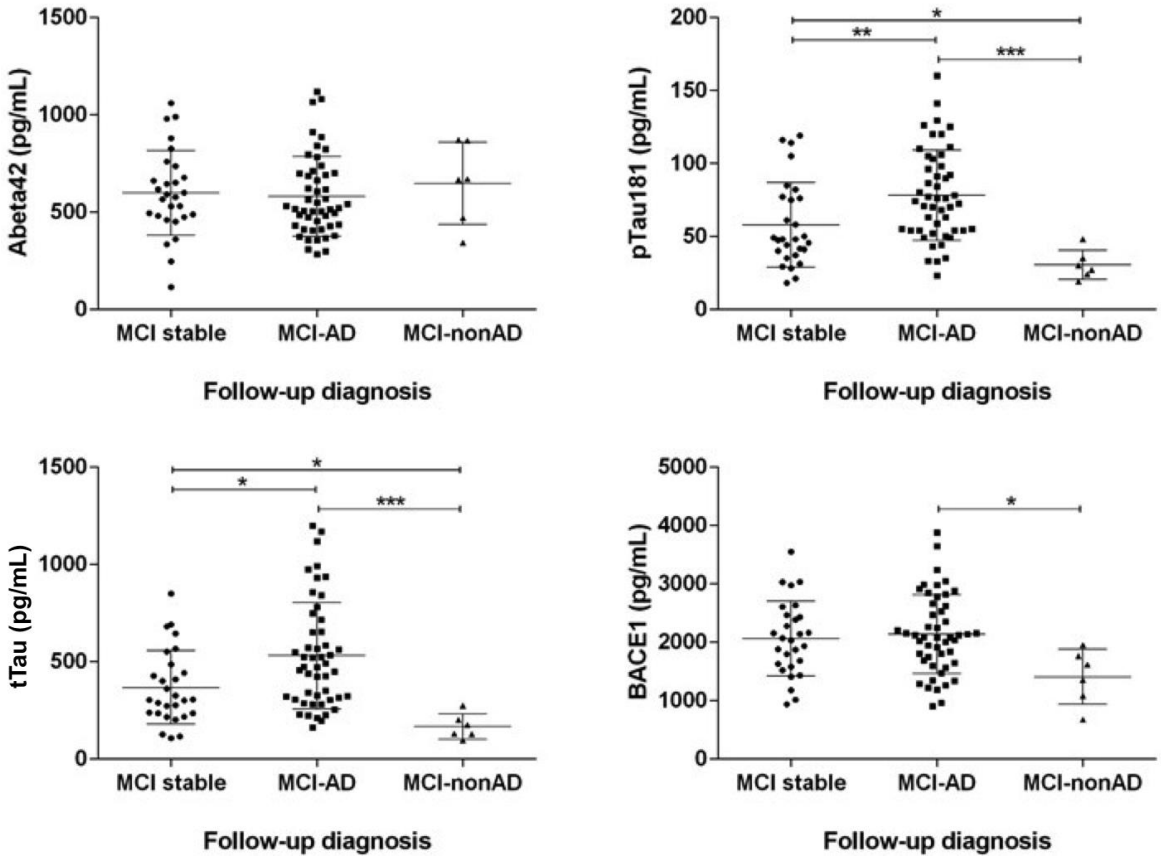

Figure 3.2 Biomarker levels according to follow-up diagnosis. *P* value indicators correspond to the values assessed with Mann-Whitney U. Post hoc analysis provided significant differences between groups: * *P*<.0167; ** *P*<.005; *** *P*<.001. Abbreviations: Abeta42, amyloid-β protein of 42 amino-acids; BACE1, β-site amyloid precursor protein cleavage enzyme 1; CO, cognitively healthy; MCI, mild cognitive impairment; MCI-AD, MCI progressing to AD dementia; MCI-nonAD, MCI progressing to dementia other than AD; pTau181, tau protein phosphorylated at threonine 181; tTau, total tau protein.

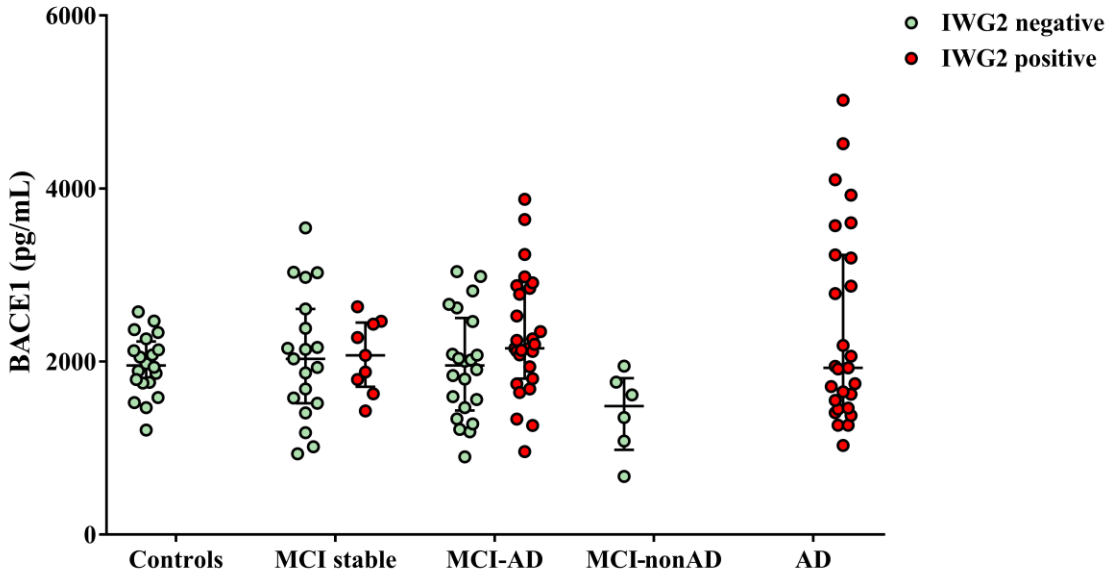

Figure 3.3 CSF BACE1 levels according to follow-up diagnosis and biomarker profile as established by the IWG-2 criteria [33]. Abbreviations: AD, Alzheimer's disease; BACE1, β-site amyloid precursor protein cleavage enzyme 1; MCI, mild cognitive impairment; MCI-AD, MCI progressing to AD dementia; MCI-nonAD, MCI progressing to dementia other than AD.

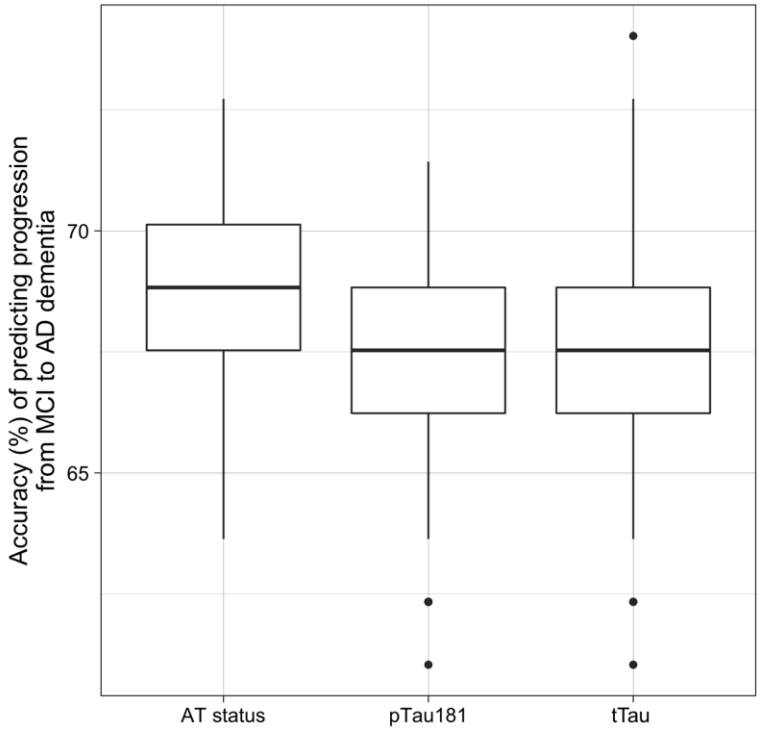

Figure 3.4 Accuracy (%) of predicting progression to AD dementia by baseline biomarker levels of the entire MCI population, calculated by performing 100x 10-fold cross-validation. Abbreviations: AD, Alzheimer's disease; AT status, amyloid and tau status; MCI, mild cognitive impairment; pTau181, tau protein phosphorylated at threonine 181; tTau, total tau protein.

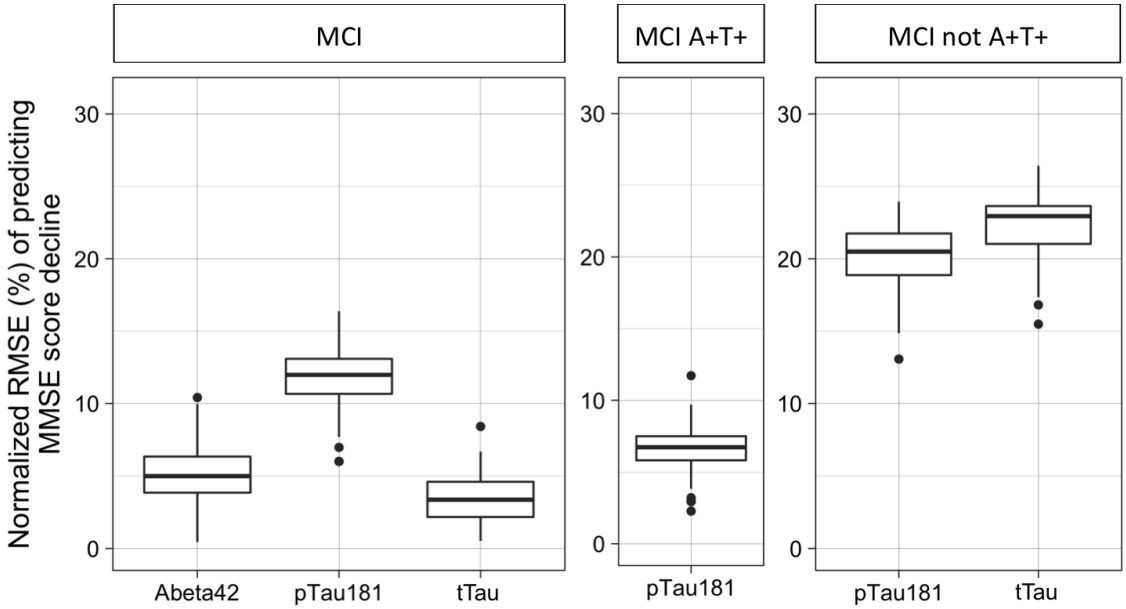

Figure 3.5 RMSE of the prediction of MMSE decline by baseline biomarker levels in the entire MCI population, the A+T+ MCI subjects and in MCI subjects which were not A+T+, based on 100x 10-fold cross validation. RMSE values were normalized for biomarker unit. Abbreviations: Abeta42, amyloid-β protein of 42 amino-acids; MCI, mild cognitive impairment; pTau181, tau protein phosphorylated at threonine 181; RMSE, root-mean-square error; tTau, total tau protein.

Chapter 4. Tau pathology

4.1 Cerebrospinal fluid $pTau_{181}$: biomarker for improved differential dementia diagnosis

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Contribution: Selection of patients, collection of patient data, data quality control, statistical analyses, interpretation of results, literature review, writing of paper

Abstract

The goal of this study is to investigate the value of tau phosphorylated at threonine 181 ($pTau_{181}$) in the AD CSF biomarker panel for differential dementia diagnosis in autopsy confirmed AD and non-AD patients.

The study population consisted of 140 autopsy confirmed AD and 77 autopsy confirmed non-AD dementia patients. CSF concentrations of $A\beta_{1-42}$, tTau, and $pTau_{181}$ were determined with single analyte ELISA-kits (INNOTEST®, Fujirebio, Ghent, Belgium). Diagnostic accuracy was assessed through receiver operating characteristic (ROC) curve analyses to obtain area under the curve (AUC) values and to define optimal cutoff values to discriminate AD from pooled and individual non-AD groups. ROC curve analyses were only performed on biomarkers and ratios that differed significantly between the groups. Pairwise comparison of AUC values was performed by means of DeLong tests.

The $A\beta_{1-42}/pTau_{181}$ ratio (AUC=0.770) performed significantly better than $A\beta_{1-42}$ (AUC=0.677, *P*=.004), tTau (AUC=0.592, *P*<.001), and $A\beta_{1-42}$/tTau (AUC=0.678, *P*=.001), while $pTau_{181}$ (AUC=0.720) performed significantly better than tTau (AUC=0.592, *P*<.001) to discriminate between AD and the pooled non-AD group. When comparing AD and the individual non-AD diagnoses, $A\beta_{1-42}/pTau_{181}$ (AUC=0.894) discriminated AD from frontotemporal lobar degeneration significantly better than $A\beta_{1-42}$ (AUC=0.776, *P*=.020) and tTau (AUC=0.746, *P*=.004), while $pTau_{181}$/tTau (AUC=0.958) significantly improved the differentiation between AD and Creutzfeldt-Jakob disease as compared to $A\beta_{1-42}$ (AUC=0.688, *P*=.004), tTau (AUC=0.874, *P*=.040), and $A\beta_{1-42}/pTau_{181}$ (AUC=0.760, *P*=.003).

In conclusion, this study demonstrates $pTau_{181}$ is an essential component of the AD CSF biomarker panel and combined assessment of $A\beta_{1-42}$, tTau, and $pTau_{181}$ renders, to present date, the highest diagnostic power to discriminate between AD and non-AD dementias.

Introduction

As explained in the general introduction of this PhD thesis, decreased $A\beta_{1\text{-}42}$ and increased tTau and/or $pTau_{181}$ concentrations are found in the CSF of AD patients compared with controls. However, when compared with non-AD dementia patients, the differences are less obvious as the concentrations in patients with non-AD dementias are generally intermediate compared with those found between controls and AD patients, thus pointing to an overlap between AD and non-AD patients, especially in dementia with Lewy bodies (DLB) and to a lesser extent in frontotemporal dementia (FTD), vascular dementia (VaD), and Creutzfeldt-Jakob's disease (CJD) [98]. This overlap may partly be explained by the presence of mixed pathologies as well as the low sensitivity and specificity of the clinical diagnosis as most biomarker studies rely on clinically diagnosed patients.

The goal of this study is to investigate the value of $pTau_{181}$ in the AD CSF biomarker panel for differential dementia diagnosis in autopsy confirmed AD and non-AD patients.

Materials and methods

Study population

In brief, the study population consisted of 140 and 77 CSF samples from dementia patients with pathologically confirmed diagnoses of AD and non-AD, respectively. All CSF samples were selected from the Biobank, Institute Born-Bunge, Antwerp, Belgium. Samples from 173 dementia patients were collected in the Memory Clinic of the Hospital Network Antwerp (ZNA, Antwerp, Belgium) between January 1992 and May 2008, whereas samples from 44 dementia patients were collected in referring centers between April 1992 and May 2005. The study was approved by the local ethics committee (CME Middelheim) and all subjects gave written informed consent.

Pathological criteria

All pathological diagnoses were established according to standard neuropathological criteria by the same neuropathologist (JJM). Although the

neuropathologist was blinded for the CSF biomarker data, he had access to all neuroimaging data and the clinical files of the patients included. For the diagnosis of AD, VaD (n= 18) and DLB (n=24) the neuropathological criteria of Montine, Phelps [138] were applied. Frontotemporal lobar degeneration (FTLD) (n=17) was neuropathologically diagnosed according to the Cairns criteria [139] and Mackenzie criteria [140, 141]. CJD (n=13) was diagnosed according to the criteria of Markesbery [142]. Mixed dementia (MXD) was diagnosed when the patient fulfilled the neuropathological criteria of AD in combination with minor pathology suggestive of cerebrovascular disease (n=12), dementia with Lewy bodies (n=1) or Parkinson's disease (n=1). For statistical analyses, the MXD group (n=14) was pooled with the AD group. The pooled non-AD group furthermore consisted of few patients with progressive supranuclear palsy (n=3), spinocerebellar ataxia (n=1) and normal pressure hydrocephalus combined with VaD (n=1). Neuropathology was performed on the right hemisphere of the brain.

CSF analyses

All subjects underwent an LP in order to collect CSF. LP was performed between the intervertebral space L3/L4 or L4/L5 [143]. CSF was sampled according to a standard protocol [53]. All samples were stored in polypropylene vials to avoid adsorption of Aβ to the wall of the vial. The samples were frozen in liquid nitrogen and stored at -80°C until analysis.

CSF concentrations of $A\beta_{1-42}$, tTau, and $pTau_{181}$ were determined with commercially available single analyte ELISA-kits (respectively, INNOTEST® β-$AMYLOID_{(1-42)}$, INNOTEST® hTAU-Ag, and INNOTEST® PHOSPHO-$TAU_{(181)}$; Fujirebio, Ghent, Belgium). A complete description of the CSF analysis has been published previously [108].

Statistical analyses

Statistical analyses were performed using SPSS 20. As most variables were not normally distributed, non-parametric tests were used. To compare gender distribution between the groups a Chi-square test was performed. Subsequently, Mann-Whitney U tests were performed to compare clinical and biomarker data

between the groups. Receiver operating characteristic (ROC) curve analyses were used to obtain area under the curve (AUC) values and to define optimal cutoff values to discriminate AD from the pooled and individual non-AD groups. ROC curve analyses were only performed on biomarkers and ratios that were significantly different ($P<0.05$), based on the Mann-Whitney U tests. The cutoff values were determined by calculating the maximal sum of sensitivity and specificity (i.e. maximizing the Youden index). In order to pairwise compare AUC values, DeLong tests were performed using the pROC package [110] in the statistical software package R (R Core Team).

Systematic review

To be able to compare the results of this study, a systematic review on the diagnostic accuracy of $pTau_{181}$ for differential dementia diagnosis was performed. A PubMed search (until May 2015) was performed using the following terms: (Cerebrospinal fluid OR CSF) AND diagnos* AND (Alzheimer* OR AD OR dementia) AND (tau OR beta amyloid OR abeta) AND (sensitivity OR specificity). Only publications in the English language were evaluated. Subsequently, relevant publications were searched for in reference lists. Publications were included when: (a) their aim was to improve the diagnostic accuracy of diagnosis of dementia by means of CSF biomarkers, (b) AD patients and pooled non-AD patients or patients with DLB, FTD, VaD and/or CJD were included, (c) $pTau_{181}$ together with $A\beta_{1\text{-}42}$ and/or tTau was measured in CSF, and (d) diagnostic accuracy values were reported (AUC, sensitivity and/or specificity). Publications comparing only AD to healthy control subjects were not considered.

Results

Table 4.1 shows the demographic, clinical, and biomarker data of the studied population. The AD and non-AD groups were not age-matched. However, based on co-variate analyses, confounding effects of age on differences in biomarker concentrations were excluded. Therefore, no corrections for age were included in the subsequent analyses. Boxplots of the individual biomarkers and ratios are presented in Figure 4.1.

The diagnostic powers to discriminate between AD and non-AD of the individual biomarkers and ratios that were significantly different are shown in Table 4.2. Based on the DeLong tests (Table 4.3) the AUC of the $A\beta_{1-42}/pTau_{181}$ ratio was significantly different from those of $A\beta_{1-42}$, tTau, and $A\beta_{1-42}$/tTau, while the AUC of $pTau_{181}$ differed significantly from the AUC of tTau.

When comparing AD and the different non-AD diagnoses, the $A\beta_{1-42}/pTau_{181}$ ratio was significantly different in every differential diagnosis (Table 4.4). This also held true for $pTau_{181}$, except for AD versus CJD. On the other hand, $pTau_{181}$/tTau was found to be significantly different when comparing AD to CJD.

The diagnostic powers to discriminate between AD and the different non-AD diagnoses of the individual biomarkers and ratios that differed significantly are shown in Table 4.5. Based on the DeLong tests (Table 4.6) the $A\beta_{1-42}/pTau_{181}$ ratio performed significantly better than $A\beta_{1-42}$ and tTau to discriminate AD from FTLD, while the AUC of $pTau_{181}$/tTau was significantly better than those of $A\beta_{1-42}$, tTau, and $A\beta_{1-42}/pTau_{181}$ to differentiate between AD and CJD.

The results of the systematic review are summarized in Supplementary table 4.1. Only results comparing AD to non-AD, FTLD, DLB, CJD and/or VaD were included in this table.

Discussion

The goal of this study was to investigate the value of $pTau_{181}$ in the AD biomarker panel for differential dementia diagnosis. First of all, the ratio of $A\beta_{1-42}/pTau_{181}$ was shown to have a significantly higher diagnostic power than $A\beta_{1-42}$, tTau, and the $A\beta_{1-42}$/tTau ratio, while $pTau_{181}$ was found to perform significantly better than tTau to discriminate between AD and non-AD dementia. This clearly signifies the importance of $pTau_{181}$ in the biomarker panel for differential dementia diagnosis. Our results are in line with previously reported findings of (combinations with) $pTau_{181}$ having most power to discriminate between AD and non-AD dementias [53, 62, 99, 102, 144-155].

However, in contrast to former studies performed in clinically diagnosed AD and pooled non-AD dementia patients [99, 144, 148-151], the AUC, sensitivity and

specificity of neither $pTau_{181}$ nor $A\beta_{1-42}/pTau_{181}$ reached the minimal level of 0.80, as established by the Consensus Report of the Working Group on Molecular and Biochemical Markers of AD [156]. This is probably not due the accuracy of the diagnoses used in this study, as autopsy confirmation was used. A possible explanation of the discrepancy in accuracy levels between this study and former studies could be the composition of the non-AD groups. As shown in this study, the accuracy levels of, for example, AD vs. FTLD are substantially higher than those of AD vs. DLB. Therefore, if a non-AD group is primarily composed of FTLD patients, the AUC levels may be higher than when DLB patients prevail in the non-AD group.

When focusing on the discrimination between AD and FTLD, our results showed that the diagnostic power of $A\beta_{1-42}/pTau_{181}$ was significantly higher than those of $A\beta_{1-42}$ and tTau. These results confirm earlier studies performed in clinically diagnosed AD and FTLD patients [102, 145-147, 153].

With regard to the differentiation between AD and CJD, the diagnostic power of $pTau_{181}$/tTau was significantly higher than those of $A\beta_{1-42}$, tTau, and $A\beta_{1-42}/pTau_{181}$. Our results confirm those of former studies performed in clinically diagnosed AD and CJD patients and partly performed in autopsy confirmed cases [157-160].

In these latter two comparisons with individual non-AD groups the AUCs did reach the minimal level of 0.80. This indicates that the pathophysiological variability in the pooled non-AD group lowers the diagnostic accuracy of the CSF biomarkers.

It should be noted that the ratios and other combinations of the AD CSF biomarkers should be used with care. Due to (pre-)analytical issues [161] concentrations differ exceedingly between laboratories. External quality controls and reference material might be able to reduce this variability, which would enable the general use of the same cutoff that was validated in a multicenter setting. At this moment, cutoffs for individual biomarkers as well as ratios and other combinations should be validated in-house before they can be used in clinical practice [162, 163].

In order to further increase diagnostic accuracy, other biomarkers should be included in the biomarker panel in the future. Examples of such possible fluid biomarkers for features of Aβ processing in AD are β-site APP cleaving enzyme-1 (BACE1) activity [124, 125, 127, 128, 164-166], soluble amyloid precursor protein

(sAPP) α and β [127, 166-173], and Aβ oligomers [174-182]. Some fluid biomarkers that are still being investigated seem more specific for non-AD dementias and could also increase diagnostic accuracy when added to the biomarker panel. Examples of possible non-AD biomarkers are TAR DNA-binding protein 43 (TDP-43) [183-185], TPD-43 phosphorylated at S409 (pTDP-43) [185], and progranulin [186-188] for FTLD, α-synuclein [189-193], and neurosin [194] for DLB, metalloproteinases-9 for VaD [195, 196], and total CSF prion protein for CJD [197]. For reviews on these biomarkers, see [198-202]. Most of these biomarkers need extensive validation as well as validated ready-to-use analytical methods before they can be used in combination with $A\beta_{1\text{-}42}$, tTau, and $pTau_{181}$ for differential dementia diagnosis in clinical practice.

Another highly promising approach is combining fluid biomarkers and imaging, such as magnetic resonance imaging (MRI), and positron emission tomography (PET) imaging. Several studies have shown that combinations of fluid and imaging biomarkers render higher diagnostic power than these modalities alone [203-207].

In conclusion, this study demonstrates $pTau_{181}$ is a fundamental component of the AD biomarker panel and the combined assessment of $A\beta_{1\text{-}42}$, tTau, and $pTau_{181}$ renders, to present date, the highest diagnostic power to discriminate between AD and non-AD dementias. New biomarkers more specifically targeted at non-AD dementia pathology should further increase diagnostic power in the future.

Table 4.1 Demographic, clinical, and biomarker data of the study population.

	AD	**non-AD**	***P* value**
N (M / F)	140 (71 / 69)	77 (45 / 32)	.275
Age at sampling (years)	76 (71-85)	72 (65-76)	.001
MMSE (/30)	14 (9-19) (n=98)	16 (9-21) (n=51)	.228
Years between sampling and death	0.0 (0.0-2.5)	0.0 (0.0-2.0)	.452
$A\beta_{1\text{-}42}$ (pg/mL)	361 (264-485)	514 (369-695)	<.001
tTau (pg/mL)	581 (335-872)	379 (242-787)	.025
$pTau_{181}$ (pg/mL)	73.2 (51.6-100.0)	45.0 (31.9-65.9)	<.001
$A\beta_{1\text{-}42}$/tTau	0.682 (0.399-1.100)	1.273 (0.719-2.257)	<.001
$A\beta_{1\text{-}42}/pTau_{181}$	4.982 (3.174-7.802)	10.535 (6.522-16.711)	<.001
$pTau_{181}$/tTau	0.138 (0.113-0.171)	0.141 (0.090-0.158)	.094

All data are median values with 25th and 75th quartiles between brackets, except for N. To compare gender distribution between the groups a Chi-square test was performed, while Mann-Whitney U tests were used to compare clinical and biomarker data between the groups. Abbreviations: AD; Alzheimer's disease; non-AD; dementia not due to Alzheimer's disease; MMSE; Mini-Mental State Examination; $A\beta_{1\text{-}42}$; amyloid-β peptide of 42 amino acids; tTau; total tau protein; $pTau_{181}$; tau phosphorylated at threonine 181.

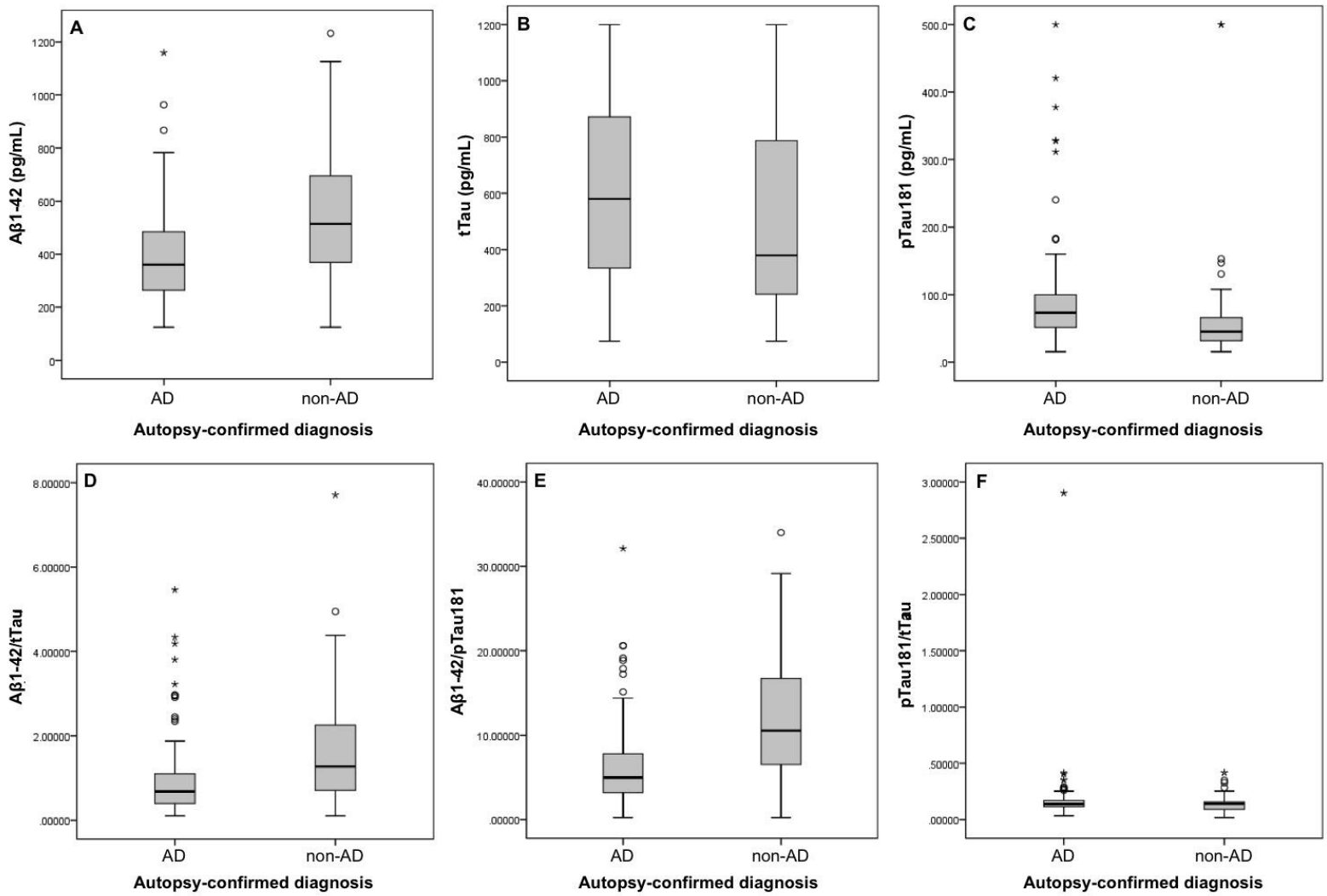

Figure 4.1 Boxplots of the individual biomarkers and ratios, comparing AD and non-AD. A. Aβ1-42; B. tTau; C. pTau181; D. Aβ1-42/tTau; E. Aβ1-42/pTau181; F. pTau181/tTau. Abbreviations: AD; Alzheimer's disease; non-AD; dementia not due to Alzheimer's disease; Aβ1-42; amyloid-β peptide of 42 amino acids; tTau; total tau protein; pTau181; tau phosphorylated at threonine 181.

Table 4.2 Diagnostic power of the significantly different individual biomarkers and ratios to discriminate between AD and non-AD, measured by ROC curve analyses.

AD vs. non-AD	AUC	95% CI	cutoff	sens (%)	spec (%)
$A\beta_{1\text{-}42}$	0.677	0.597-0.757	500.27	79.3	53.2
tTau	0.592	0.508-0.675	472.35	62.1	63.6
$pTau_{181}$	0.720	0.648-0.792	50.35	77.9	61.0
$A\beta_{1\text{-}42}$/tTau	0.678	0.601-0.755	1.08	75.0	57.1
$A\beta_{1\text{-}42}/pTau_{181}$	0.770	0.703-0.837	9.11	82.9	59.7

Abbreviations: AD; Alzheimer's disease; non-AD; dementia not due to Alzheimer's disease; AUC; area under the curve; CI; confidence interval; sens; sensitivity; spec; specificity; $A\beta_{1\text{-}42}$; amyloid-β peptide of 42 amino acids; tTau; total tau protein; $pTau_{181}$; tau phosphorylated at threonine 181.

Table 4.3 *P* values of pairwise comparisons of AUC values of the ROC curve analyses to discriminate between AD and non-AD, using DeLong tests.

AD vs. non-AD	$pTau_{181}$	$A\beta_{1\text{-}42}/pTau_{181}$
$A\beta_{1\text{-}42}$	.450	**.004**
tTau	**<.001**	**<.001**
$A\beta_{1\text{-}42}$/tTau	.290	**.001**
$A\beta_{1\text{-}42}/pTau_{181}$	.100	*NA*

Abbreviations: $A\beta_{1\text{-}42}$; amyloid-β peptide of 42 amino acids; tTau; total tau protein; $pTau_{181}$; tau phosphorylated at threonine 181; NA, not applicable.

Table 4.4 *P* values of pairwise comparisons of the individual biomarkers and ratios, measured by Mann-Whitney U tests.

	AD vs. FTLD	**AD vs. DLB**	**AD vs. CJD**	**AD vs. VaD**
$A\beta_{1\text{-}42}$	<.001	.068	.025	.078
tTau	.001	.051	<.001	.019
$pTau_{181}$	<.001	.011	.081	.001
$A\beta_{1\text{-}42}$/tTau	<.001	.008	.054	.010
$A\beta_{1\text{-}42}/pTau_{181}$	<.001	.002	.002	.003
$pTau_{181}$/tTau	.096	.232	<.001	.932

Abbreviations: AD; Alzheimer's disease; FTLD; frontotemporal lobar degeneration; DLB; dementia with Lewy bodies; CJD; Creutzfeldt-Jakob disease; VaD; vascular dementia; $A\beta_{1\text{-}42}$; amyloid-β peptide of 42 amino acids; tTau; total tau protein; $pTau_{181}$; tau phosphorylated at threonine 181.

Table 4.5 Diagnostic power of the significantly different individual biomarkers and ratios to discriminate between AD and individual non-AD diagnoses, measured by ROC curve analyses.

	AUC	95% CI	cutoff	sens (%)	spec (%)
AD vs. FTLD					
$A\beta_{1-42}$	0.776	0.652-0.900	385.31	57.1	88.2
tTau	0.746	0.654-0.838	423.00	67.9	82.4
$pTau_{181}$	0.810	0.710-0.910	47.25	81.4	76.5
$A\beta_{1-42}$/tTau	0.863	0.794-0.931	0.97	70.1	94.1
$A\beta_{1-42}/pTau_{181}$	0.894	0.823-0.965	9.77	86.4	82.4
AD vs. DLB					
$pTau_{181}$	0.664	0.539-0.788	59.05	65.7	70.8
$A\beta_{1-42}$/tTau	0.670	0.539-0.802	0.80	60.7	75.0
$A\beta_{1-42}/pTau_{181}$	0.694	0.565-0.824	8.46	80.0	58.3
AD vs. CJD					
$A\beta_{1-42}$	0.688	0.521-0.855	440.12	66.4	69.2
tTau	0.874	0.775-0.973	>1200	84.3	92.3
$A\beta_{1-42}/pTau_{181}$	0.760	0.634-0.886	6.84	67.9	84.6
$pTau_{181}$/tTau	0.958	0.925-0.991	0.1030	84.3	100.0
AD vs. VaD					
tTau	0.670	0.534-0.807	467.93	62.1	72.2
$pTau_{181}$	0.733	0.599-0.867	49.85	78.6	66.7
$A\beta_{1-42}$/tTau	0.687	0.569-0.804	0.72	56.4	77.8
$A\beta_{1-42}/pTau_{181}$	0.718	0.598-0.838	5.30	55.7	77.8

Abbreviations: AD; Alzheimer's disease; FTLD; frontotemporal lobar degeneration; DLB; dementia with Lewy bodies; CJD; Creutzfeldt-Jakob disease; VaD; vascular dementia; AUC; area under the curve; CI; confidence interval; sens; sensitivity; spec; specificity; $A\beta_{1-42}$; amyloid-β peptide of 42 amino acids; tTau; total tau protein; $pTau_{181}$; tau phosphorylated at threonine 181.

Table 4.6 *P* values of pairwise comparisons of AUC values of the ROC curve analyses to discriminate between AD and individual non-AD diagnoses, using DeLong tests.

	$pTau_{181}$	**$A\beta_{1-42}/pTau_{181}$**	**$pTau_{181}/tTau$**
AD vs. FTLD			
$A\beta_{1-42}$	.700	**.020**	*NA*
tTau	.120	**.004**	*NA*
$A\beta_{1-42}$/tTau	.280	.280	*NA*
AD vs. DLB			
$A\beta_{1-42}$/tTau	.890	.360	*NA*
AD vs. CJD			
$A\beta_{1-42}$	*NA*	.327	**.004**
tTau	*NA*	.220	**.040**
$A\beta_{1-42}/pTau_{181}$	*NA*	*NA*	**.003**
AD vs. VaD			
tTau	.370	.600	*NA*
$A\beta_{1-42}$/tTau	.600	.610	*NA*

Abbreviations: AD; Alzheimer's disease; FTLD; frontotemporal lobar degeneration; DLB; dementia with Lewy bodies; CJD; Creutzfeldt-Jakob disease; VaD; vascular dementia; $A\beta_{1-42}$; amyloid-β peptide of 42 amino acids; tTau; total tau protein; $pTau_{181}$; tau phosphorylated at threonine 181; NA, not applicable.

Supplementary table 4.1. Diagnostic accuracy of the individual biomarkers and combinations of biomarkers in previously published studies. Abbreviations: AD; Alzheimer's disease; $A\beta_{1-42}$; amyloid-β peptide of 42 amino acids; AUC; area under the curve; CJD; Creutzfeldt-Jakob disease; DLB; dementia with Lewy bodies; FTD; frontotemporal dementia; non-AD; dementia not due to Alzheimer's disease; NR; not reported; $pTau_{181}$; tau phosphorylated at threonine 181; sens; sensitivity; spec; specificity; tTau; total tau protein; VaD; vascular dementia.

Study	Groups	N	CSF biomarkers	AUC	cutoff	sens (%)	spec (%)
AD vs. non-AD							
Maddalena, Papassotiropoulos [150]	AD	51	$A\beta_{1-42}$	0.731	0.49 ng/mL	78.0	70.0
	non-AD	30	$pTau_{181}$	0.710	35 pg/mL	73.0	63.0
			$pTau_{181}/A\beta_{1-42}$	0.801	83 pg/mL	80.0	73.0
Olsson, Vanderstichele [208]*	AD	78	$A\beta_{1-42}$	*NR*	515 pg/mL	91.0	75.0
	non-AD	128	tTau	*NR*	436 pg/mL	83.0	89.0
			$pTau_{181}$	*NR*	87.3 pg/mL	72.0	95.0
Lewczuk, Kornhuber [209]*	AD	53	$A\beta_{1-42}$	*NR*	197.5 pg/mL	75.5	60.0
	non-AD	15	tTau	*NR*	86 pg/mL	66.0	80.0
			$pTau_{181}$	*NR*	47.9 pg/mL	77.4	73.3
Engelborghs, De Vreese [53]	Definite AD	51	$A\beta_{1-42}$	*NR*	*NR*	*NR*	*NR*
	Definite non-AD	15	tTau	*NR*	*NR*	*NR*	*NR*
			$pTau_{181}$	*NR*	*NR*	*NR*	*NR*
			Model $A\beta_{1-42}$ + $pTau_{181}$	0.941	2.03	80.0	93.0
Welge, Fiege [99]	AD	44	$A\beta_{1-42}$	0.774	0.458 ng/mL	86.0	64.0
	non-AD	87	tTau	0.711	0.266 ng/mL	87.0	48.0
			$pTau_{181}$	0.874	0.065 ng/mL	87.0	83.0
			$A\beta_{1-42}$/tTau	0.773	1.451	92.0	60.0
			$A\beta_{1-42}/pTau_{181}$	0.894	8.157	95.0	75.0
Yakushev, Bartenstein [144]	AD	24	tTau	0.730	440 pg/mL	54.0	92.0
	non-AD	13	$pTau_{181}$	0.910	65 pg/mL	71.0	100
Gabelle, Dumurgier [148]	AD	272	$A\beta_{1-42}$	0.760	519 pg/mL	81.6	71.4
	non-AD	370	tTau	0.840	362 pg/mL	81.2	78.4
			$pTau_{181}$	0.870	61 pg/mL	76.8	88.4
			$A\beta_{1-42}$/tTau	0.860	2.48	86.7	78.7
			$A\beta_{1-42}/pTau_{181}$	0.880	15.1	85.2	84.0
			Model $A\beta_{1-42}$ + $pTau_{181}$	0.900	-0.37	84.5	86.5

Supplementary table 4.1 (cont.)

Study	Groups	N	CSF biomarkers	AUC	cutoff	sens (%)	spec (%)
AD vs. non-AD							
Shea, Chu [149]	AD	24	$A\beta_{1\text{-}42}$	0.690	301.6 pg/mL	63.0	83.0
	non-AD	12	tTau	0.670	370.2 pg/mL	83.0	58.0
			$pTau_{181}$	0.740	31.51 pg/mL	100	42.0
			$A\beta_{1\text{-}42}$/tTau	0.740	1.54	96.0	50.0
			$A\beta_{1\text{-}42}/Tau_{181}$	0.800	6.87	79.0	75.0
Duits, Teunissen [151]	AD	631	$A\beta_{1\text{-}42}$	0.800	550 pg/mL	82.0#	72.0
	non-AD	267	tTau	0.790	375 pg/mL	82.0#	63.0
			$pTau_{181}$	0.810	52 pg/mL	86.0#	59.0
			$A\beta_{1\text{-}42}$ + tTau and/or $pTau_{181}$ abnormal			74.0#	81.0
			≥ 2 of 3 biomarkers abnormal			86.0#	65.0
			tTau/$A\beta_{1\text{-}42}$	0.850	0.71	85.0#	75.0
			$pTau_{181}/A\beta_{1\text{-}42}$	0.860	0.11	85.0#	80.0
			Hulstaert model [210] ($A\beta_{1\text{-}42}$ + tTau)	0.850	1	93.0#	65.0
			Mulder model [211] ($A\beta_{1\text{-}42}$ + tTau)	0.850	1	93.0#	63.0
			Mattsson model [212] ($A\beta_{1\text{-}42}$ + tTau + $pTau_{181}$)	0.860	1	80.0#	80.0
			Schoonenboom model [213] ($A\beta_{1\text{-}42}$ + $pTau_{181}$)	0.860	1	91.0#	72.0
Seeburger, Holder [214]	Definite AD	92	$A\beta_{1\text{-}42}$	*NR*	463 pg/mL	84.0	100
	Definite non-AD	16	tTau	*NR*	438 pg/mL	73.0	94.0
			$pTau_{181}$	*NR*	44 pg/mL	90.0	60.0
			tTau/$A\beta_{1\text{-}42}$	*NR*	0.798	92.0	100
			$pTau_{181}/A\beta_{1\text{-}42}$	*NR*	0.131	88.0	100
AD vs. FTD							
Schoonenboom, Pijnenburg [147]	AD	47	$A\beta_{1\text{-}42}$	0.860	413 pg/mL	85.0	75.0
	FTD	28	tTau	0.813	377 pg/mL	85.0	74.0
			$pTau_{181}$	0.866	54 pg/mL	85.0	82.0
Blasko, Lederer [146]	AD	23	$A\beta_{1\text{-}42}$	*NR*	*NR*	*NR*	*NR*
	FTD	5	tTau	*NR*	*NR*	*NR*	*NR*
			$pTau_{181}$	*NR*	*NR*	*NR*	*NR*
			$pTau_{181}/A\beta_{1\text{-}42}$	0.900	*NR*	86.0	80.0
Gabelle, Roche [102]	AD	52	$A\beta_{1\text{-}42}$	0.750	464 pg/mL	79.0	62.0
	FTD	34	tTau	0.880	448 pg/mL	88.0	82.0
			$pTau_{181}$	0.950	58 pg/mL	91.0	88.0
			Hulstaert model [210] ($A\beta_{1\text{-}42}$ + tTau)	0.870	0.66	88.0	86.0

Supplementary table 4.1 (cont.)

Study	Groups	N	CSF biomarkers	AUC	cutoff	sens (%)	spec (%)
AD vs. FTD							
de Souza, Lamari [145]	AD	60	$A\beta_{1\text{-}42}$	0.817	292.1 pg/mL	68.3	85.2
	FTD	27	tTau	0.832	458 pg/mL	70.0	88.9
			$pTau_{181}$	0.851	62.5 pg/mL	83.3	85.2
			$tTau/A\beta_{1\text{-}42}$	0.926	1.23	95.0	85.2
			$pTau_{181}/A\beta_{1\text{-}42}$	0.942	0.211	91.7	92.6
Irwin, Trojanowski [215] *	Definite AD	30	$A\beta_{1\text{-}42}$	0.874	*NR*	*NR*	*NR*
	Definite FTD	10	tTau	0.941	*NR*	*NR*	*NR*
			$pTau_{181}$	0.889	*NR*	*NR*	*NR*
			$tTau/A\beta_{1\text{-}42}$	0.989	0.34	*NR*	*NR*
			$pTau_{181}/A\beta_{1\text{-}42}$	0.956	*NR*	*NR*	*NR*
Ewers, Mattsson [153] *	AD	167	$A\beta_{1\text{-}42}$	*NR*	*NR*	85.0	77.0
	FTD	39	tTau	*NR*	*NR*	*NR*	*NR*
			$pTau_{181}$	*NR*	*NR*	*NR*	*NR*
			Model $A\beta_{1\text{-}42}$ + $pTau_{181}$	*NR*	*NR*	85.0	85.0
AD vs. DLB							
Vanderstichele, De Vreese [62]	AD	94	$A\beta_{1\text{-}42}$	*NR*	*NR*	*NR*	*NR*
	DLB	60	tTau	*NR*	*NR*	*NR*	*NR*
			$pTau_{181}$ ¶	*NR*	61 pg/mL	80.0	79.0
Wada-Isoe, Kitayama [216]	AD	24	$A\beta_{1\text{-}42}$	*NR*	*NR*	*NR*	*NR*
	DLB	22	$pTau_{181}$	*NR*	46.3 pg/mL	68.2	82.4
			$pTau_{181}/A\beta_{1\text{-}42}$	*NR*	13.3	72.7	70.6
Aerts, Esselink [152]	AD	44	$A\beta_{1\text{-}42}$	0.650	482 pg/mL	62.0	65.0
	DLB	21†	tTau	0.950	294 pg/mL	90.4	90.0
			$pTau_{181}$	0.920	67 pg/mL	81.0	95.0
			Model $A\beta_{1\text{-}42}$ + tTau + $pTau_{181}$	0.960	0.42	92.9	90.0
Ewers, Mattsson [153] *	AD	167	$A\beta_{1\text{-}42}$	*NR*	*NR*	85.0	42.0
	DLB	26	tTau	*NR*	*NR*	*NR*	*NR*
			$pTau_{181}$	*NR*	*NR*	*NR*	*NR*
			Model $A\beta_{1\text{-}42}$ + $pTau_{181}$	*NR*	*NR*	85.0	77.0
AD vs. CJD							
Bahl, Heegaard [160]	AD	49	tTau	*NR*	500 pg/mL	67.0	100
	CJD	21	$pTau_{181}$	*NR*	*NR*	*NR*	*NR*
			$pTau_{181}$/tTau	*NR*	0.040	86.0	98.0

Supplementary table 4.1 (cont.)

Study	Groups	N	CSF biomarkers	AUC	cutoff	sens (%)	spec (%)
AD vs. VaD							
de Jong, Jansen [154]	AD	61‡	$A\beta_{1-42}$	*NR*	520 pg/mL	82.0	76.0
	VaD	25‡	tTau	*NR*	321 pg/mL	80.0	76.0
			$pTau_{181}$	*NR*	68.5 pg/mL	75.0	95.0
			$tTau/A\beta_{1-42}$	*NR*	1.2	82.0	92.0
			$pTau_{181}/A\beta_{1-42}$	*NR*	10.95	95.0	90.0
Reijn, Rikkert [155]	AD	69	$A\beta_{1-42}$	0.730	540 ng/L	87.0	62.0
	VaD	26	tTau	0.810	350 ng/L	88.0	73.0
			$pTau_{181}$	0.940	75 ng/L	78.0	96.0
			$A\beta_{1-42}/pTau_{181}$	0.940	6.8	86.0	96.0
			Model $A\beta_{1-42}$ + $pTau_{181}$	0.960	-1.1	91.0	96.0
			$A\beta_{1-42}$*	0.840	213 ng/L	80.0	81.0
			tTau*	0.810	66 ng/L	81.0	85.0
			$pTau_{181}$*	0.900	51 ng/L	83.0	89.0
			$A\beta_{1-42}/pTau_{181}$*	0.930	5.7	91.0	85.0
			Model $A\beta_{1-42}$ + $pTau_{181}$*	0.930	0.11	96.0	85.0
Ewers, Mattsson [153] *	AD	167	$A\beta_{1-42}$	*NR*	*NR*	85.0	46.0
	VaD	69	tTau	*NR*	*NR*	*NR*	*NR*
			$pTau_{181}$	*NR*	*NR*	*NR*	*NR*
			Model $A\beta_{1-42}$ + $pTau_{181}$	*NR*	*NR*	85.0	59.0

* Biomarker levels determined with xMAP® technology (INNO-BIA AlzBio3, Innogenetics, Ghent).
# Sensitivity values are derived from the ROC curve analysis of AD vs. controls.
¶ The classification tree retained only $pTau_{181}$.
† DLB N; 20 for tTau.
‡ AD N; 56 and VaD N; 20 for $pTau_{181}$.

Chapter 5. Neurodegeneration

5.1 A head-to-head comparison of the predictive powers of neurodegeneration biomarkers in mild cognitive impairment

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Contribution: Selection of patients, data quality control, statistical analyses, interpretation of results, literature review, writing of paper

Abstract

Population enrichment strategies capable to maximize the occurrence of clinical progression within the time-frame of a clinical trial remains an unmet need. Here, we assessed, in patients with mild cognitive impairment, the power of neurodegeneration markers (CSF total tau, FDG PET glucose metabolism, and grey and white matter volumes) to predict progression within 24 to 48 months.

MCI (n=241) individuals from the AD Neuroimaging Initiative database with baseline cerebrospinal fluid sampling, FDG PET, MRI, and clinical assessment with at least one clinical follow-up assessment within 48 months were included. Subsequently, patients were classified as mild cognitive impairment stable (mild cognitive impairment at 48 months), fast progressors (progression at 12 or 24 months), or slow progressors (progression at 36 or 48 months while being stable at 24 months). All subjects were stratified using CSF $A\beta_{1\text{-}42}$ and $pTau_{181}$, resulting in 31 A-T-, 37 A-T+, 18 A+T-, and 155 A+T+ subjects.

Logistic regression and voxel-based logistic regression models evaluated clinical progression as a function of baseline neurodegeneration biomarker levels. Cross-validation of the regression analyses determined the power to predict (fast/slow) progression.

First, we confirmed that A+T+ drove progression to dementia. Next, in A+T+ subjects, grey matter volume was the best predictor of progression. Regional effects were observed in temporal and inferior parietal cortices. Finally, white matter volume predicted fast clinical progression in subjects with low grey matter volume and low glucose metabolism.

We conclude that a biomarker profile characterized by A(+), T(+), and N(+) is highly predictive of clinical progression in mild cognitive impairment subjects. Although grey matter atrophy is sensitive to overall clinical progression, coexistence between grey and white matter atrophy constitute best predictors of imminent clinical decline. These results have immediate application in population enrichment strategies for disease-modifying trials.

Introduction

Biomarker signatures capable for predicting upcoming progression to dementia would have an important application on reducing the enrollment of stable individuals with low probability to clinically progress during the time frame of a clinical trial. For example, as the rate of clinical decline from MCI to AD is approximately 12% per year [73], a biomarker profile capable of identifying MCI subjects on the verge of progression would allow the enrollment of the best population for a drug trial as well as increase efficiency and reducing its costs. However, it remains elusive which biomarker profile would incorporate temporal information regarding upcoming clinical progression in order to optimize the enrollment of disease-modifying trials.

As neurodegenerative changes occur later in the AD pathophysiology than amyloidosis and neurofibrillary tangles, biomarkers of neurodegeneration are expected to have a better predictive value regarding upcoming clinical progression [36]. However, it remains unclear whether the neurodegeneration markers have similar predictive powers regarding clinical progression.

Current research into biomarkers of neuronal injury focuses mainly on cortical changes, while white matter (WM) changes have been reported to be abundant in AD, especially in diffusion-tensor imaging (DTI) studies [217, 218]. WM changes in AD generally follow the anatomical pattern of cortical atrophy, supporting the theory that Wallerian degeneration may account for WM involvement in AD [217, 219]. However, as DTI is not yet a readily available biomarker for WM changes in AD, more global measures of WM damage might be more promising. In that sense, it has been show that WM atrophy predicts fast (within 2 year) progression of MCI to dementia [220-222]. WM volume should thus to be considered as a structural MRI neurodegeneration measure.

Against this background, we identified the need for a head-to-head comparison of the predictive powers of the different neurodegeneration markers, including WM volume. Consequently, we performed a longitudinal analysis in amnestic MCI individuals, investigating the power of the established neurodegeneration markers CSF total tau, [^{18}F]FDG PET, and grey matter (GM) and WM volume to predict a fast

approaching clinical progression to dementia in a 48-month follow-up period, hypothesizing that the predictive powers depend on "A/T" status [223].

Materials and methods

Database description and study participants

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. For up-to-date information, see www.adni-info.org.

For the present study, we first selected ADNI-1/GO/2 participants meeting the ADNI criteria for single-domain or multi-domain amnestic MCI, who underwent LP, [^{18}F]FDG PET, and MRI as well as neuropsychological assessments at baseline and at least one other neuropsychological assessment during a 48-month follow-up period. We selected MCI subjects according to the ADNI criteria as those who had an MMSE score equal to or greater than 24, a clinical dementia rating (CDR) of 0.5, subjective and objective memory loss, and absence of other neuropsychiatric disorders [72] (Further information regarding inclusion/exclusion criteria may be found at www.adni-info.org [accessed January 2017].)

Progression from MCI to dementia was defined as a change of CDR to a value higher than 0.5. The MCI subjects were subdivided into groups based on their cognitive trajectory: fast progression (progressed at 24-month follow-up), slow progression (progressed between 24 and 48-month follow-up), or stable (no progression at 48 months of follow-up). Stable MCI subjects thus still had a CDR score of 0.5 after 48-month follow-up. Subjects with fast progressing to dementia progressed either at the 12 or 24-month follow-up time points, while the slow progressing subjects progressed either at the 36 or 48-month follow-up time points. We only selected those subjects who progressed at 36 or 48 months who underwent clinical

assessment and were stable at 24-month and/or 36-month follow-up. This way, we ensured that subjects in the slow progression category did not actually already progress to dementia before 24-month follow-up.

In order to determine thresholds to dichotomize the subjects according to normal or abnormal neurodegeneration markers, AD dementia subjects were also included as well as cognitively healthy subjects. As the thresholds used by ADNI for CSF biomarkers were determined on autopsy-confirmed AD patients and healthy controls [224], we determined the thresholds for the other neurodegeneration markers on two extreme phenotypes of the AD spectrum as well: 106 AD dementia patients with abnormal CSF levels of both $A\beta_{1-42}$ and $pTau_{181}$ versus 54 cognitively healthy individuals with normal CSF levels of $A\beta_{1-42}$ and $pTau_{181}$. According to the ADNI criteria AD dementia subjects were defined as those who had an MMSE score between 20 and 26, a CDR of 1.0 or higher, subjective and objective memory loss, and absence of other neuropsychiatric disorders. All AD dementia patients fulfilled the NINCDS/ADRDA criteria for probable AD [4]. We selected cognitively healthy subjects defined by ADNI as those who had an MMSE score equal to or greater than 24, a CDR of 0, no objective memory loss, and absence of other neuropsychiatric disorders.

CSF analyses

The CSF levels of $A\beta_{1-42}$, $pTau_{181}$ and total tau were quantified on the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX, USA) using the INNO-BIA AlzBio3 immunoassay kit-based reagents (Innogenetics/Fujirebio, Ghent, Belgium). All of the CSF data used in this study were obtained from the ADNI file "UPENNBIOMK5-8.csv". The data were statistically rescaled based on the baseline assay analysis that was used to define the CSF $A\beta_{1-42}$ and $pTau_{181}$ thresholds [224]. (Further details can be found at www.adni-info.org [accessed January 2017].)

MRI methods

The unprocessed MRI images from the ADNI database were processed by ico**metrix** (Leuven, Belgium) using the CE-labelled and FDA-cleared software ico**brain** (formerly known as MSmetrix), to extract GM and WM volumes. To that end, the

T1-weighted MR images were segmented into WM and GM using a probabilistic model, including bias field correction [225]. Both GM and WM volumes were corrected for head size.

Subsequently, quality control of the extracted measurements was performed, consisting of a visual assessment of the segmentations of all 'outlier' measurements. 'Outliers' measurements were considered as volumes below and above the 10^{th} and 90^{th} percentiles, respectively. As a result, due to incorrect segmentation, the volumes of one MCI subject were rejected and excluded from further analyses.

[^{18}F]FDG PET

PET images were processed with an established image-processing pipeline [84]. Briefly, the PET images from the ADNI database first underwent spatial normalization to the Montreal Neurological Institute 152 standardized space. This was performed by using information obtained from transformations of PET native to the MRI native space and MRI native to the Montreal Neurological Institute 152 space. Subsequently, the [^{18}F]FDG PET standardized uptake value ratio (SUVR) maps were generated using the pons as reference region. The global brain glucose uptake was estimated by averaging the SUVR from the precuneus, prefrontal, orbitofrontal, parietal, temporal, anterior, and posterior cingulate cortices in the [^{18}F]FDG PET images.

Biomarker-based stratification of participants

For analyses purposes, the participants were stratified into four biomarker groups according to their A (amyloid) and T (tau) status, based on previously published thresholds of CSF $A\beta_{1-42}$ (<192 pg/mL) and $pTau_{181}$ (>23 pg/mL), respectively [224].

Neurodegeneration-based stratification of participants

For detailed analyses purposes, the participants were further stratified according to their N (neurodegeneration) status. The threshold for CSF total tau was published previously (>93 pg/mL) [224].

ROC curve analyses were performed to define optimal threshold for global FDG SUVR, GM and WM volume to discriminate the A+T+ AD dementia patients from the A-T- cognitively healthy individuals. Thresholds were determined by calculating the maximal sum of sensitivity and specificity (i.e. maximizing the Youden index), using R package 'OptimalCutpoints' [226]. The obtained thresholds were 1.166 for global FDG SUVR (AUC=0.860), 870 mL for GM volume (AUC=0.605), and 532 mL for WM volume (AUC=0.728).

Statistical methods

The statistical analyses were performed using RStudio (version 1.0.136) [133]. ANOVA was performed to test for significant differences of demographic characteristics and biomarker levels between groups, with Bonferroni correction for multiple comparisons.

First, to confirm if AT status predicts progression, logistic regression analyses were performed in pairwise biomarker group comparisons using progression as outcome and biomarker status as main effect and Bonferroni correction for multiple comparisons.

Next, to assess predictive powers of the neurodegeneration markers in MCI due to AD, the following analyses were performed in the A+T+ MCI group.

- In order to evaluate whether the neurodegeneration markers predict progression, a logistic regression analysis was performed using progression as outcome and biomarker levels as main effect.
- To evaluate whether the neurodegeneration markers predict slow and fast progression compared with stable, multinomial logistic regression analysis was performed using progression (i.e. stable, slow and fast progression) as outcome, biomarker levels as main covariates and stable MCI as reference group, using R package 'nnet' was used [227].
- Subsequently, to evaluate if the neurodegeneration markers predict speed of progression (fast versus slow), a logistic regression analysis was performed using progression speed as the outcome and biomarker levels as main effect.

Finally, predictive powers of the different neurodegeneration biomarkers were calculated by performing 100x 10-fold cross-validation of all the models described above, using R package 'caret' [134].

All analyses were adjusted for age, sex, years of formal education, and *APOE* ε4 carrier status.

We validated the different predictions of clinical progression to dementia described above through random forest classifiers, using R package 'randomForest' [228]. The random forest classifiers use sampling techniques to train multiple random forest predictors to calculate the final prediction. In brief, classification trees are trained on a bootstrap sample of the dataset, making successive trees independent of each other (i.e. 'bagging'). In standard trees each node is split using the best split based on all variables. However, random forests add another layer of randomness by splitting each node using a randomly chosen subset of variables at that node. Subsequently, at each bootstrap iteration, the data outside the bootstrap (i.e. 'out-of-bag') are predicted using the tree grown with the bootstrap sample. This way the error rate of the prediction can be computed. Finally, the random forest algorithm estimates the importance of a variable by calculating the degree of prediction accuracy decrease when that variable is left out (i.e. 'MeanDecreaseAccuracy') [229]. We applied 10-fold cross-validation to the random forest classifiers, in order to obtain ten MeanDecreaseAccuracy values at each tree node split.

Voxel-based logistic regression analysis

To identify the brain regions where decreased GM density determined the increased likelihood of progression to AD dementia over a 48-month period in A+T+ MCI subjects, a voxel-based logistic regression model was built to test the main effects of GM density (voxel-based morphometry, VBM) at every brain voxel on the likelihood of developing dementia, adjusting for age, gender, years of formal education, and *APOE* ε4 carrier status. The voxel-wise analyses were performed using Matlab® (http://www.mathworks.comwith/; accessed May 2017) with the 'VoxelStats' package [230].

The voxel-based statistical parametric maps were corrected for multiple comparisons using a Random Field Theory at a threshold of *P*<.001 [231].

Results

Out of 497 MCI individuals meeting the initial inclusion criteria, 241 subjects presented with the required information complying with the defined clinical progression criteria. Table 5.1 summarizes demographic and biomarker characteristics of the MCI population.

A+T+ status drives progression from MCI to dementia

Logistic regression analyses revealed that progression to dementia over 48 months (i.e. both slow and fast) is mainly driven by the A+T+ group (*P*<.001), which was confirmed by the random forest classifier analysis (Figure 5.8).

Clinical progression in the non-A+T+ groups was negligible; therefore, all subsequent analyses were performed in the A+T+ group. Table 5.2 summarizes demographic and biomarker characteristics of the A+T+ MCI subjects.

GM volume is best predictor of progression of A+T+ subjects

Logistic regression analyses revealed that progression over 48 months (i.e. both slow and fast) could be predicted by GM volume, global FDG SUVR and CSF total tau (Figure 5.1). Subsequent 100x 10-fold cross-validation revealed that GM volume had the highest predictive power (mean accuracy (±SD) = 68% (±1.6%), mean PPV (±SD) = 56% (±1.9%), mean NPV (±SD) = 77% (±1.4%)) (Figure 5.2), which was confirmed by the random forest classifier analysis confirmed (Figure 5.8).

Voxel-based logistic regression analysis showed that bilateral hippocampi, left temporal cortex, precuneus and inferior parietal cortices were the brain regions where decreased GM density determined the increased likelihood of progression to AD dementia over a 48-month period (Figure 5.3).

WM volume predicts fast progression in progressive A+T+N+ subjects

Multinomial logistic regression revealed that GM and WM volumes, and global FDG SUVR could predict fast progression (Figure 5.4). Cross-validation showed that that GM volume had the highest power to predict fast progression (mean accuracy (±SD) = 63% (±1.7%), mean PPV (±SD) = 58% (±1.3%), mean NPV (±SD) = 74% (±2.8%)) (Figure 5.5 lower panel).

The multinomial logistic regression also revealed that slow progression could be predicted by GM volume and CSF total tau (Figure 5.4). Cross-validation showed that GM volume and CSF total tau had similar predictive powers (GM volume mean accuracy (±SD) = 64% (±1.6%), mean PPV (±SD) = 66% (±1.0%), mean NPV (±SD) = 55% (±6.0%); CSF total tau mean accuracy (±SD) = 64% (±1.5%), mean PPV (±SD) = 65% (±0.7%), mean NPV (±SD) = 59% (±7.1%)) (Figure 5.5 upper panel).

As GM volume had high predictive powers for both slow and fast progression, additional analyses were performed to evaluate which neurodegeneration marker has the highest power to predict slow and fast progression in progressive subjects. As a result, logistic regression showed that only WM volume could discriminate slow and fast progression in progressive subjects (Figure 5.4), which was confirmed by the random forest classifier analysis (Figure 5.8). Cross-validation showed that the power to predict fast progression of WM volume was 59% (±2.5%) mean accuracy (±SD), 43% (±3.1%) mean PPV (±SD), and 70% (±2.3%) mean NPV (±SD) (Figure 5.6).

Additional logistic regression in subjects dichotomized according to normal and abnormal GM volume, global FDG SUVR, or CSF total tau revealed that WM volume only predicts fast progression in the subjects with abnormal GM volume and global FDG SUVR. Cross-validation showed that the power to predict fast progression of WM volume was higher in A+T+GM+ compared with A+T+FDG+ subjects (64% (±2.6%) vs. 63% (±2.7%) mean accuracy (±SD), 52% (±2.8%) vs. 40% (±4.6%) mean PPV (±SD), and 75% (±2.4%) vs. 73% (±2.3%) mean NPV (±SD) in A+T+GM+ and A+T+FDG+ subjects, respectively) (Figure 5.7).

Discussion

In this study we performed a thorough head-to-head comparison of the power of the neurodegeneration markers CSF total tau, [^{18}F]FDG PET, and GM and WM volume to predict upcoming clinical progression from MCI to dementia, assuming the interdependency between neuronal degeneration and positive "AT" status as the driver of clinical progression on MCI [223]. Our main findings were (1) amnestic MCI subjects with abnormal CSF levels of both $A\beta_{1\text{-}42}$ and $pTau_{181}$ had the highest rate of clinical progression to dementia in comparison to all other biomarker groups; (2) within the subjects with abnormal CSF levels of both $A\beta_{1\text{-}42}$ and $pTau_{181}$, GM volume predicted progression to dementia; and (3) WM volume could subsequently best predict fast progression in those A+T+ subjects with abnormal GM volume. The impact of these findings in patient selection of clinical trials targeting MCI due to AD might be significant, as the quantification of WM and GM atrophy might constitute a rapid and affordable screening procedure.

The first main finding confirms previous studies reporting synergy between amyloid and tau as a driving force for the development of AD dementia, both in MCI [80, 84] and cognitively healthy subjects [81-83, 85]. Further analyses in the A+T+ MCI subjects revealed that GM volume is the best predictor of progression in the A+T+ MCI subjects, compared with [^{18}F]FDG PET and CSF total tau. Based on previous studies, we rather expected [^{18}F]FDG PET to present with the highest predictive power [232-234]. According to the biomarker model of Jack and colleagues changes in CSF total tau happen early in the pathophysiological process of AD, while lower glucose metabolism occurs later, followed by brain atrophy [36]. Hence, the predictive power of the different biomarkers depends in large extent on the disease phase of the included subjects and the follow-up time. In our study, we investigated progression to dementia in a 48-month follow-up period. Although this is considered as a long follow-up, it might still be "late" in the disease course, possibly explaining why GM volume outperformed [^{18}F]FDG PET in our study.

In line with this hypothesis, we found that WM volume best predicted the progression to dementia in A+T+GM+ subjects in 24 months. As it had no predictive

power in the A+T+GM- population, the WM atrophy in A+T+GM+ probably reflects Wallerian degeneration in MCI subjects.

Overall, our results revealed that the neurodegeneration markers, most specifically GM and WM volume, have an additional value to AT in predicting MCI clinical progression. Interestingly, we found that WM volume predicts fast progression in those A+T+ MCI subjects who are likely to progress due to low GM volume. This finding extends previous studies conducted on MCI subjects with short follow-up time, demonstrating that global WM and corpus callosum volumes are reduced in fast progressing MCI subjects [220-222]. Our finding points to WM atrophy following GM atrophy as being a late state of disease pathophysiology with higher specificity for imminent clinical progression and might provide evidence for the probable sequential order where synergy between amyloidosis and neurofibrillary tangles lead to neuronal damage, which in turn leads to axonal decay [36].

Besides adding insights into imminent clinical decline, our study also sheds light on more distant decline by analyzing 48-month follow-up while including the entire set of established neurodegeneration markers. Previous studies conducted with shorter follow-up periods and only a subset of markers have proposed that combining neurodegeneration markers with measures of amyloid improves prediction of progression [203, 235-244]. By performing a head-to-head comparison we were able to identify global GM volume, mainly driven by changes in temporal and inferior parietal regions, as the best predictor of overall clinical progression during 48 months in A+T+ MCI subjects. Interestingly, the temporal and inferior parietal cortices found here to be associated with an increased likelihood of clinical progression in A+T+ MCI subjects, are well known as vulnerable brain regions in predementia AD patients [245].

The interpretation of these results should take into consideration some methodological limitations. First, vascular load was not included in our statistical framework. Hence, we cannot assess its influence on the WM volume loss observed in this study. However, ADNI systematically excludes subjects with neurological syndromes that might cause cognitive impairment, like the presence of substantial vascular burden. We might thus assume that subjects enrolled in ADNI only present

with age-related WM changes. It has been shown that atrophy of the corpus callosum in AD does not correlate with age-related WM changes [222], so we conclude that the WM volume loss we found in progressive A+T+ MCI subjects is largely independent from age-related WM changes.

Furthermore, as our study focused on the value of the biomarkers, we do not provide explanations of the neuropathological processes underlying biomarker changes. Hence, more elaborate studies such as DTI studies comparing WM volume to DTI measures should be performed to locate and quantify WM changes leading to WM volume loss in A+T+N+ MCI subjects. In addition, as neurodegeneration markers are known to be the least specific for AD, we anticipate studies validating the predictive power of neurodegeneration markers in non-AD diseases as well.

Unfortunately, our attempt to replicate our findings in cognitively healthy subjects failed, probably due to the high number of subjects "converting back" to normal and thus introducing a bias in the progression/stable classification. Longer regular follow-up of these subjects is necessary to reliably determine time of progression and separate progressive and stable subjects, and test the predictive power of the ATN biomarkers in cognitively healthy individuals.

From a clinical trial perspective, these results could greatly improve subject selection for population enhancement. The combination of $A\beta_{1\text{-}42}$, $pTau_{181}$, and GM and WM volumes could identify those MCI subjects most likely to progress to dementia within 24 months, or those with less abundant brain damage more likely to benefit from treatment.

In conclusion, the association between the A, T, and N markers is highly important to predict clinical progression to AD dementia in MCI subjects. Although cortical changes are sensitive to overall clinical progression, the present findings support the coexistence of GM and WM atrophy as a late state of disease pathophysiology with higher specificity for imminent clinical decline in subjects presenting with amyloid and tau abnormalities.

Table 5.1 Demographics and biomarker characteristics of the MCI population.

	MCI A-T-	**MCI A-T+**	**MCI A+T-**	**MCI A+T+**	***P* value**
N (% of total population)	31 (13)	37 (15)	18 (8)	155 (64)	-
Female, N (%)	10 (32)	19 (51)	4 (22)	63 (41)	.160
Age, years	74.3 (6.7)	70.0 (7.9)	71.0 (6.2)	73.4 (6.8)	.024
APOE ε4, no. (%)	6 (19)	6 (16)	9 (50)[b]	112 (72)[a,b]	<.001
Education, years	16.8 (2.0)	16.1 (2.6)	16.3 (2.9)	15.9 (2.7)	.308
MMSE at baseline, /30	27.9 (1.7)	28.0 (1.8)	28.6 (0.9)	27.2 (1.9)[c]	.002
CSF $A\beta_{1\text{-}42}$, pg/mL	227.3 (24.6)	229.2 (27.4)	145.7 (36.1)[a,b]	133.0 (22.3)[a,b]	<.001
CSF $pTau_{181}$, pg/mL	16.8 (3.8)	35.9 (9.0)[a]	17.7 (4.1)[b]	55.9 (25.9)[a,c]	<.001
CSF total tau, pg/mL	44.9 (17.0)	66.3 (21.6)	49.8 (19.8)	121.0 (55.3)[a,b,c]	<.001
Global FDG, SUVR	1.265 (0.143)	1.289 (0.143)	1.183 (0.123)[b]	1.176 (0.122)[a,b]	<.001
GM volume, mL	855.8 (42.6)	867.5 (33.9)	850.1 (36.2)	850.1 (47.8)	.123
WM volume, mL	538.3 (32.2)	542.4 (49.1)	535.7 (43.6)	535.6 (40.7)	.820
Follow-up groups, no. (%)					
MCI stable	24 (77)	34 (92)	14 (78)	58 (37)	-
MCI progressive	7 (23)	3 (8)	4 (22)	97 (63)[a,b,c]	<.001

All data are mean values with standard deviation between brackets, except when indicated otherwise. Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; SD, standard deviation; CSF, cerebrospinal fluid; $A\beta_{1\text{-}42}$, amyloid-β peptide of 42 amino acids; MMSE, mini-mental state examination; $pTau_{181}$, tau phosphorylated at threonine 181; SUVR, standardized uptake value ratio.
Note: *P* values indicate the values assessed with analyses of variance for each variable except gender, *APOE* ε4, and diagnosis at follow-up, where a contingency chi-square was performed. Post hoc analysis provided significant differences between groups: [a]from MCI A-T-; [b]from MCI A-T+; [c]from MCI A+T-.

Table 5.2 Demographics and biomarker characteristics of the A+T+ MCI population.

	MCI stable	Slow progressors	Fast progressors	*P* value
N (% of A+T+ population)	58 (37)	35 (23)	62 (40)	-
Female, N (%)	23 (40)	14 (40)	26 (42)	.964
Age, years	73.0 (7.1)	72.4 (6.3)	74.3 (6.7)	.377
APOE ε4, no. (%)	37 (64)	27 (77)	48 (77)	.191
Education, years	15.4 (2.6)	16.5 (2.6)	16.0 (2.7)	.125
MMSE at baseline, /30	27.7 (1.8)	27.2 (1.9)	26.8 (1.8)[a]	.019
CSF $A\beta_{1\text{-}42}$, pg/mL	136.7 (23.2)	132.2 (19.0)	130.1 (23.0)	.267
CSF $pTau_{181}$, pg/mL	49.8 (25.1)	64.2 (31.6)[a]	56.9 (21.8)	.031

All data are mean values with standard deviation between brackets, except when indicated otherwise. Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; SD, standard deviation; CSF, cerebrospinal fluid; $A\beta_{1\text{-}42}$, amyloid-β peptide of 42 amino acids; MMSE, mini-mental tate examination; $pTau_{181}$, tau phosphorylated at threonine 181.
Note: *P* values indicate the values assessed with analyses of variance for each variable except gender, *APOE* ε4, and diagnosis at follow-up, where a contingency chi-square was performed. Post hoc analysis provided significant differences between groups: [a]from MCI stable.

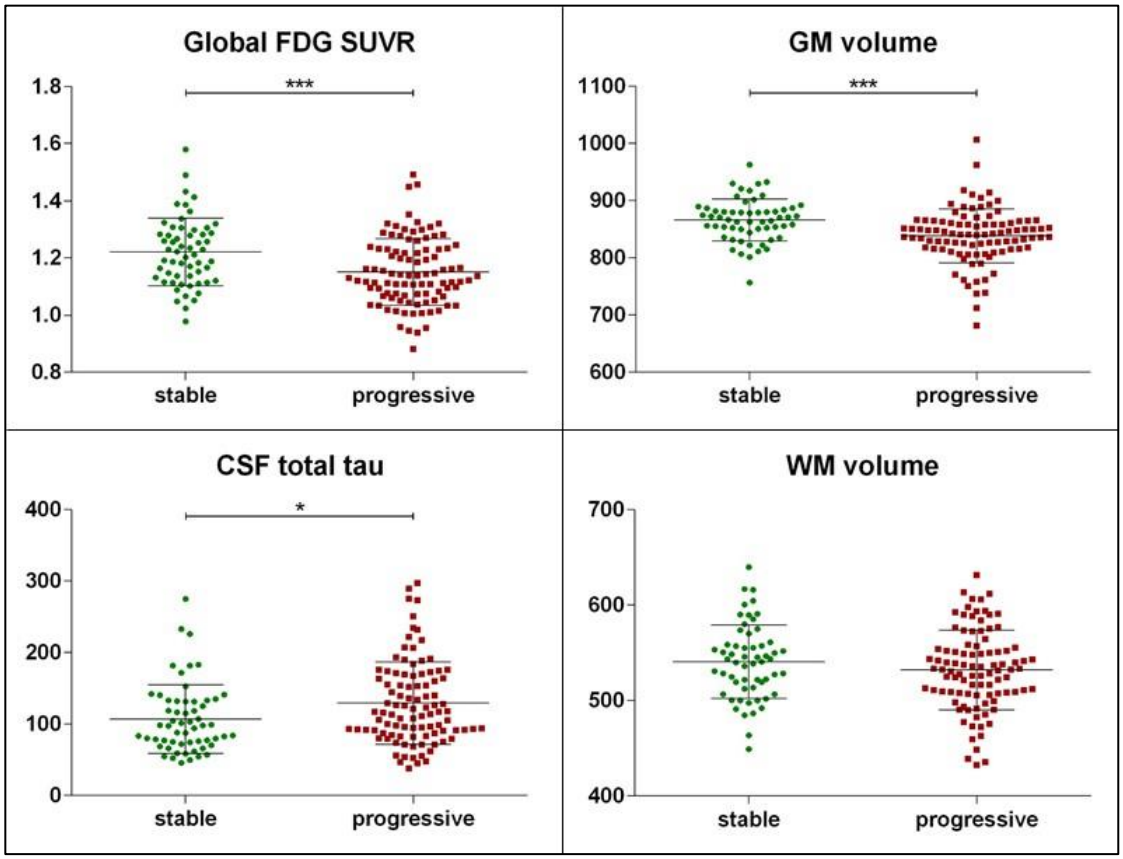

Figure 5.1 Baseline global FDG SUVR, CSF total tau, and GM and WM volume in stable and progressive A+T+ aMCI. Clinical progression to dementia over 48 months (i.e. both slow and fast) was observed in those individuals with high levels of cortical neurodegeneration. Abbreviations: aMCI, amnestic mild cognitive impairment; FDG, fluorodeoxyglucose; SUVR, standardized uptake value ratio; GM, grey matter; CSF, cerebrospinal fluid; WM, white matter.

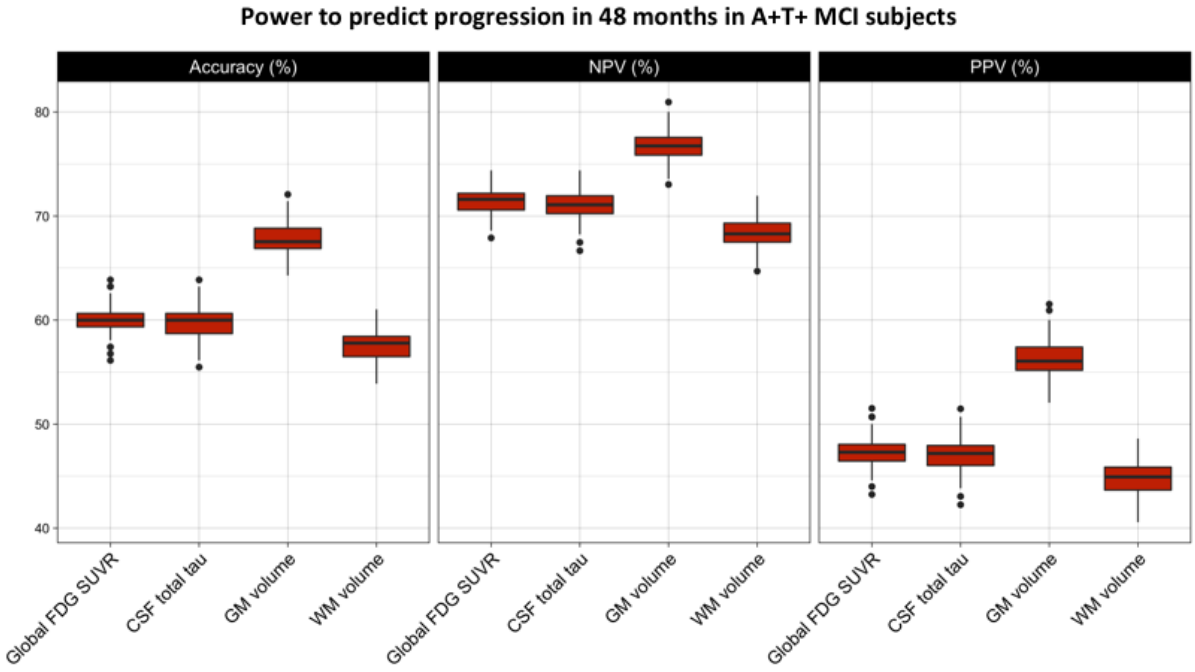

Figure 5.2 Power (accuracy, NPV, and PPV values) of biomarkers of neurodegeneration for predicting progression, calculated by performing 100x 10-fold cross-validation. GM volume had the highest power to predict clinical progression to dementia over 48 months. Abbreviations: NPV, negative predictive value; PPV, positive predictive value; FDG, fluorodeoxyglucose; SUVR, standardized uptake value ratio; GM, grey matter; CSF, cerebrospinal fluid; WM, white matter.

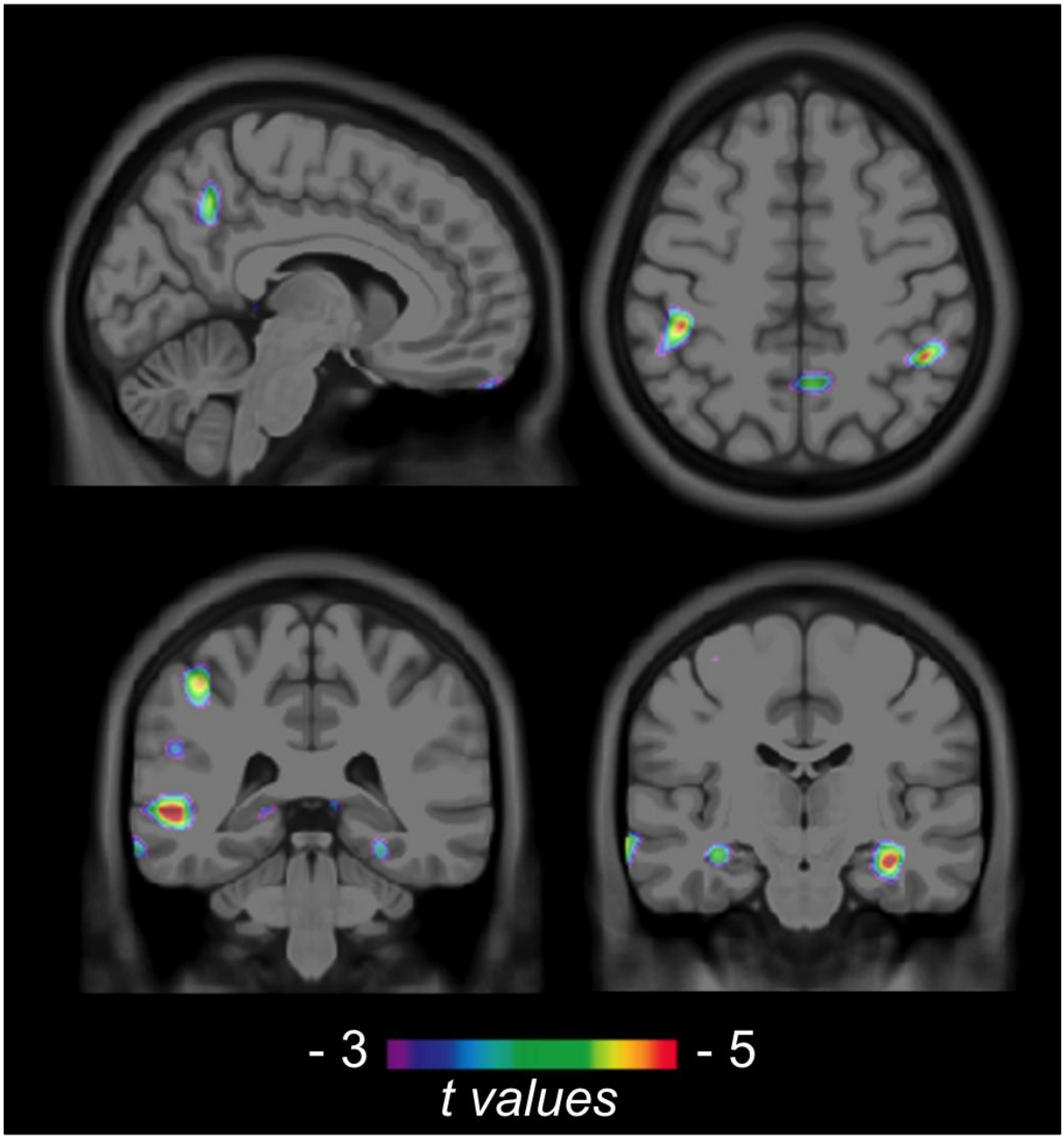

Figure 5.3 T-statistical parametric map, after correcting for multiple comparisons (random field theory at *P*<·001), overlaid in a structural magnetic resonance scan. Decreased grey matter density in temporal and inferior parietal cortices predict clinical progression to dementia over 48 months (i.e. both slow and fast) in A+T+ amnestic MCI subjects. Note reduced baseline levels of grey matter density in bilateral hippocampi, left temporal, precuneus and inferior parietal cortices in those who progress to dementia in the subsequent 48 months.

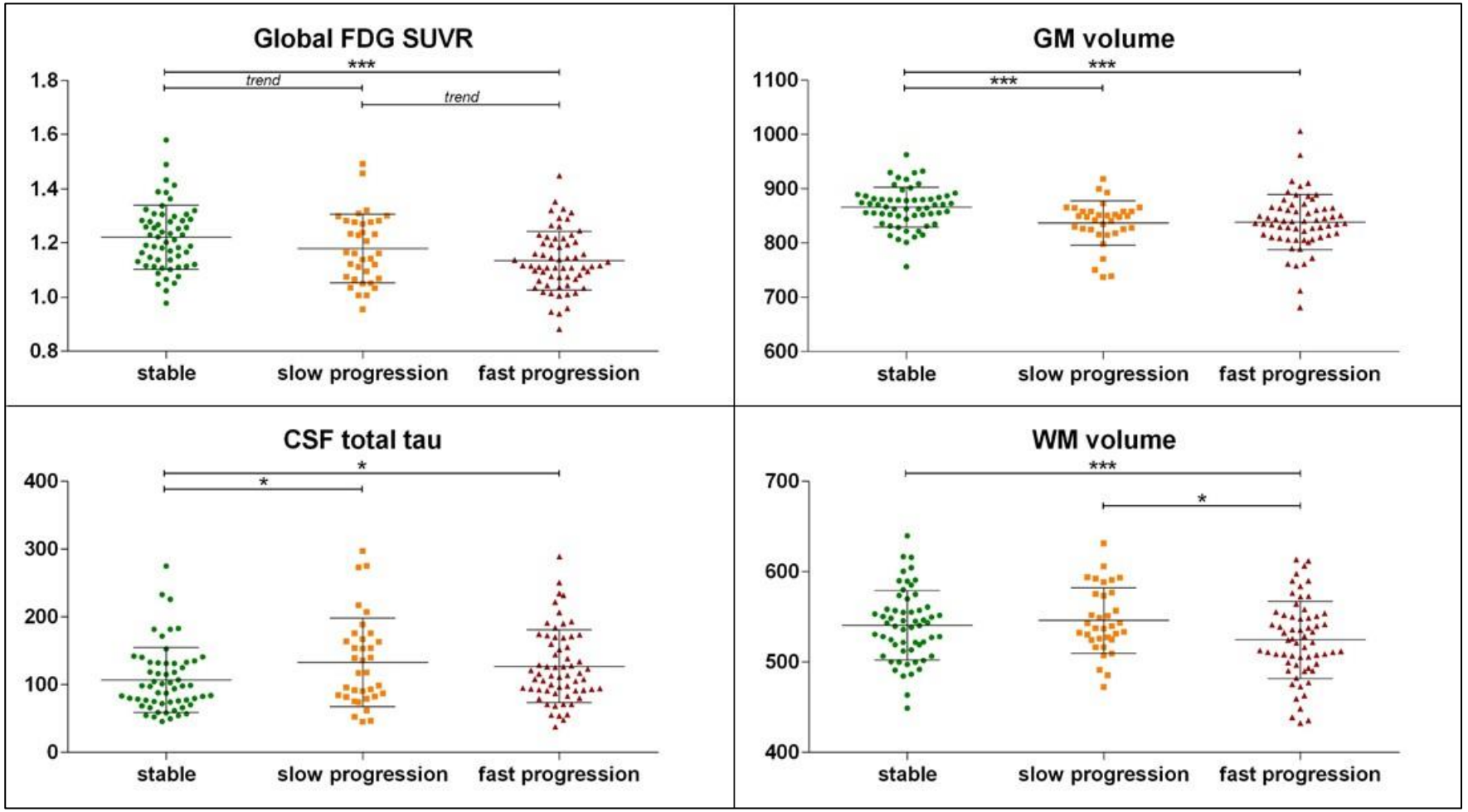

Figure 5.4 Baseline global FDG SUVR, CSF total tau, and GM and WM volume in stable, slow and fast progressive A+T+ aMCI subjects. Slow clinical progression to dementia was observed in those individuals with low GM volume and high levels of CSF total tau, while fast clinical progression was observed in those individuals with low GM and WM volume, and low global FDG SUVR. Only WM volume predicted fast progression in progressive A+T+ amnestic MCI to dementia. Abbreviations: FDG, fluorodeoxyglucose; SUVR, standardized uptake value ratio; GM, grey matter; CSF, cerebrospinal fluid; WM, white matter.

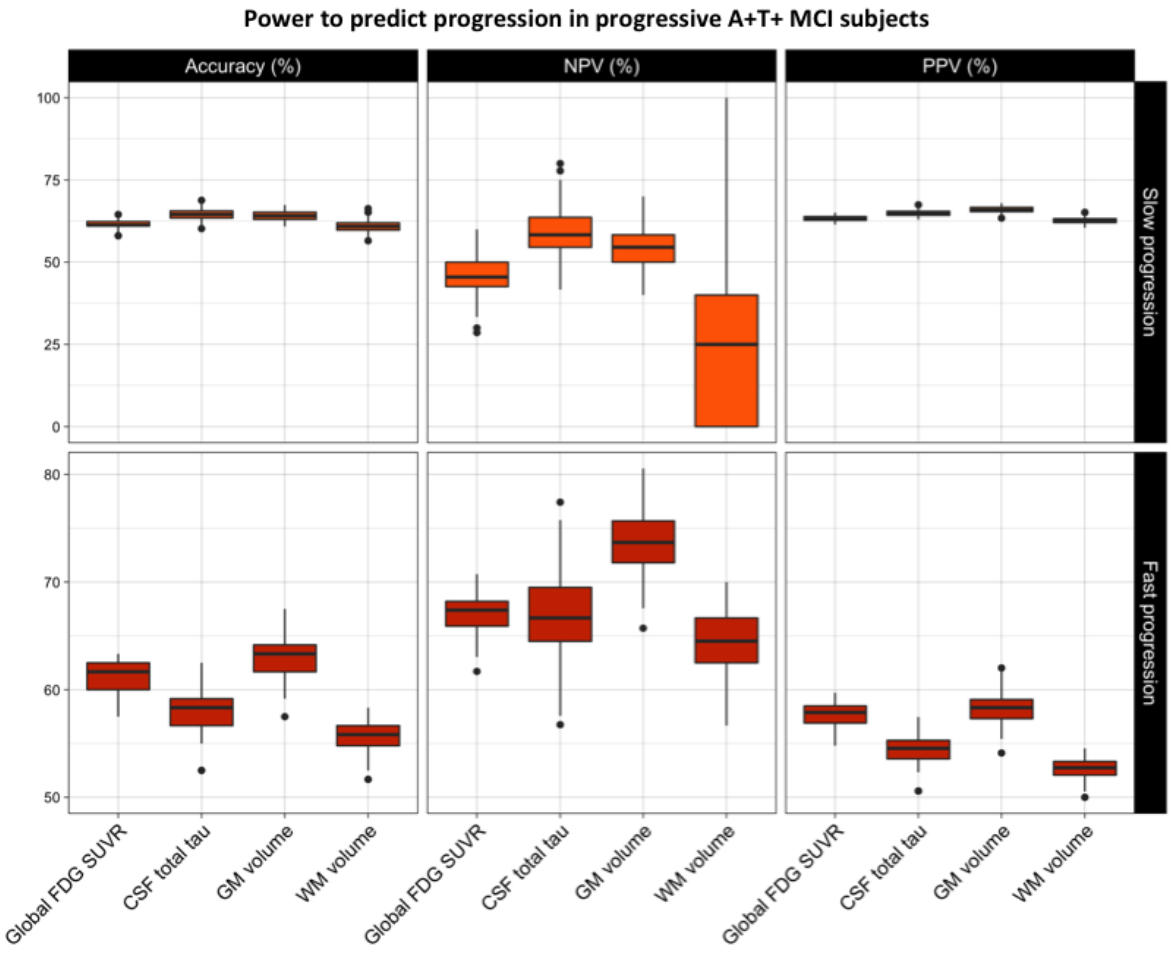

Figure 5.5 Power (accuracy, NPV, and PPV values) of biomarkers of neurodegeneration for predicting slow and fast progression compared with stable aMCI, calculated by performing 100x 10-fold cross-validation. GM volume and CSF total tau had similar effects to predict slow progression to dementia (upper panel). GM volume had the highest power to predict fast clinical progression to dementia (lower panel). Abbreviations: NPV, negative predictive value; PPV, positive predictive value; FDG, fluorodeoxyglucose; SUVR, standardized uptake value ratio; GM, grey matter; CSF, cerebrospinal fluid; WM, white matter.

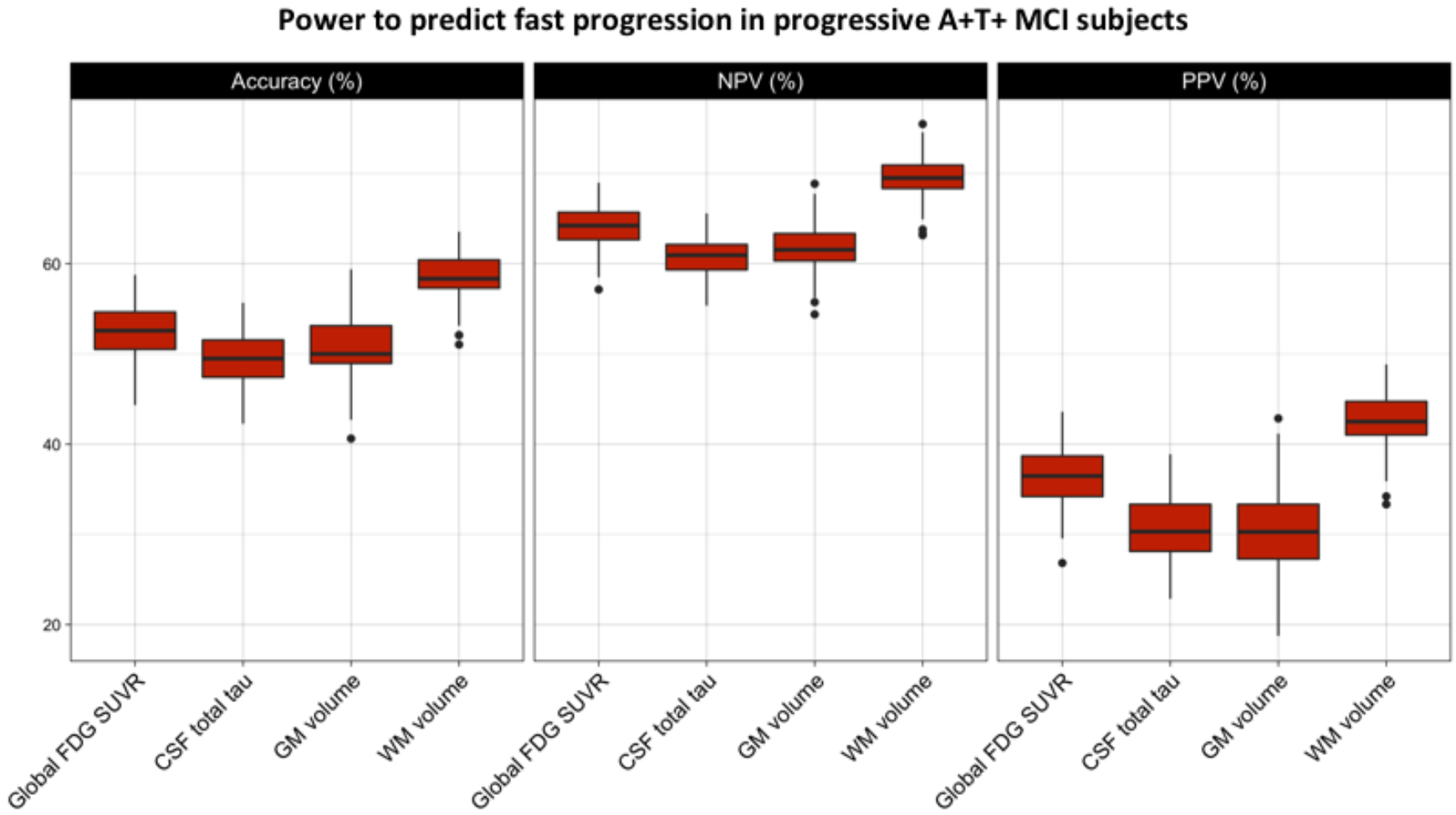

Figure 5.6 WM volume had the highest power to predict fast clinical progression to dementia in progressive A+T+ amnestic MCI, based on accuracy, NPV, and PPV values from 100x 10-fold cross-validation. Abbreviations: NPV, negative predictive value; PPV, positive predictive value; FDG, fluorodeoxyglucose; SUVR, standardized uptake value ratio; GM, grey matter; CSF, cerebrospinal fluid; WM, white matter.

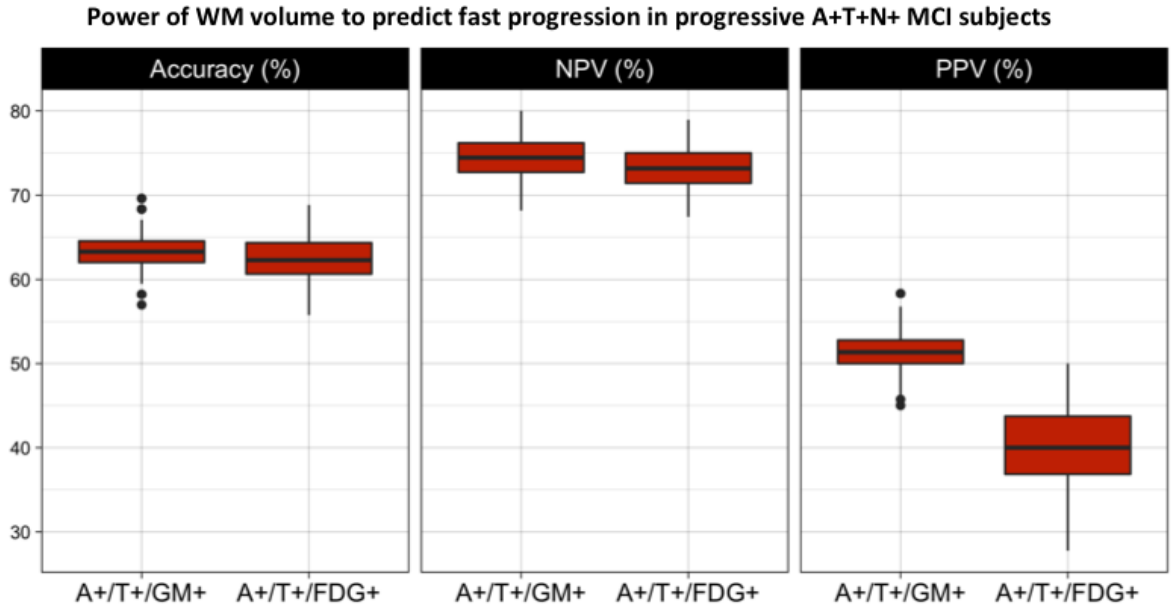

Figure 5.7 WM volume had the highest power to predict fast clinical progression to dementia in progressive A+T+GM+ amnestic MCI, based on accuracy, NPV, and PPV values from 100x 10-fold cross-validation. Abbreviations: WM, white matter; N, neurodegeneration marker; NPV, negative predictive value; PPV, positive predictive value; GM, grey matter; FDG, fluorodeoxyglucose.

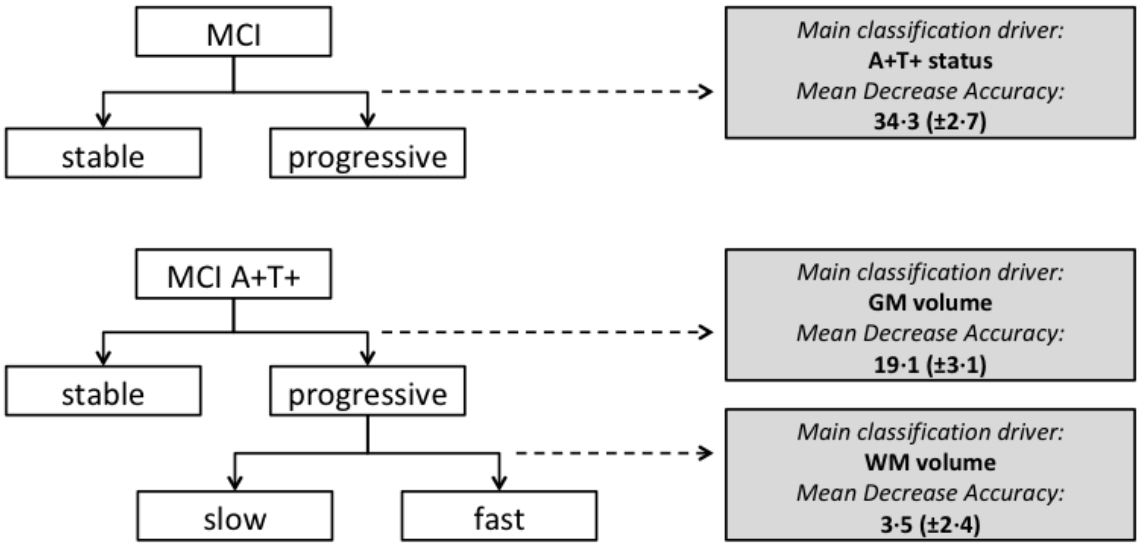

Figure 5.8 The step-wise classification of amnestic MCI subjects was validated using machine learning and summarized in this figure. The initial classification into stable and progressive MCI subjects was driven by A+T+ MCI subjects. Within these subjects GM volumes predicts clinical progression, while WM volume predicts speed of progression within the progressive A+T+ MCI subjects. Abbreviations: MCI, mild cognitive impairment; GM, grey matter; WM, white matter.

5.2 Cerebrospinal fluid neurofilament light: useful biomarker to predict progression in mild cognitive impairment subjects without biomarker changes of amyloid and neurofibrillary tangles

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Manuscript in preparation.

Contribution: Selection of patients, collection of patient data and samples, data quality control, statistical analyses, interpretation of results, literature review, writing of paper

Abstract

Though the combination of the core AD biomarkers are highly accurate for AD, only $A\beta_{1\text{-}42}$ is regarded as a really early marker and thus new additional biomarkers would be of great value for early-stage diagnosis and prognosis. The aim of this study was to compare the power to predict future progression to AD dementia and cognitive decline in MCI patients of a panel of well-studied CSF biomarker candidates to the predictive power of the core AD CSF biomarkers $A\beta_{1\text{-}42}$, $pTau_{181}$, and tTau.

The biomarkers that were included were neurofilament light (NFL), neurogranin, visinin-like protein-1 (VLP1), fatty acid-binding protein 3, and YKL40, as they have all been shown to be, to some extent, predictive of cognitive decline in AD in previous studies.

Logistic and linear regression models evaluated clinical progression to AD dementia and MMSE decline, respectively, as a function of baseline biomarker levels. Subsequent cross-validation determined the power to predict progression and MMSE decline.

Of all the candidate biomarkers, only NFL was predictive of future progression to AD dementia. It's prediction accuracy levels, however, were lower than AT status in the total MCI population. Yet, as NFL was the only predictor of progression to AD dementia in the heterogeneous MCI group without an A+T+ biomarker profile, NFL was shown to have an added value in this MCI subpopulation. Regarding prediction of MMSE decline, VLP1 was the only predictive candidate biomarker. However, as it's predictive power was never higher than $pTau_{181}$ and tTau, VLP1 was found to have no added value.

The predictive power of the candidate biomarkers in comparison to the core AD CSF biomarkers $A\beta_{1\text{-}42}$, $pTau_{181}$, and tTau was limited. The exception is NFL, which had an added value to predict progression to AD dementia of the heterogeneous MCI group without an A+T+ profile.

Introduction

Though the combination of the core AD biomarkers are highly accurate for AD, only $A\beta_{1-42}$ is regarded as a really early marker [115] and thus new additional biomarkers would be of great value for early-stage diagnosis but also to measure target engagement of candidate drugs in clinical trials targeting early pathological changes. During the past decade, many novel CSF biomarkers were discovered that might be promising to predict progression to AD dementia and have been proposed as candidates to be added to the core AD CSF biomarker panel. However, whether they have an added value on top of the core AD CSF biomarkers still remains unclear. Hence, the aim of this study was to compare the powers to predict future progression to AD dementia and cognitive decline in MCI patients of a panel of well-studied CSF biomarker candidates to the predictive powers of the core AD CSF biomarkers $A\beta_{1-42}$, $pTau_{181}$, and tTau.

The biomarkers included in this study have all been shown to be, to some extent, predictive of cognitive decline in AD in previous studies. They comprized neurofilament-light (NFL) [246-249], neurogranin (Ng) [249-252], visinin-like protein-1 (VLP1) [253-259], fatty acid-binding protein 3 (FABP3) [260-265], and YKL-40 [253, 266, 267]. This study was particularly designed as a pilot study to identify biomarkers that should be considered for a prospective validation study.

Materials and methods

For a detailed description of the materials and methods used in this study, please refer to the Materials and methods section of Chapter 3.2. It should be noted that outliers of NFL and YKL40 in the MCI population were excluded from regression analyses.

CSF analyses

The core AD CSF biomarkers $A\beta_{1-42}$, $pTau_{181}$, and tTau were analyzed with INNOTEST® assays (INNOTEST® β-AMYLOID$_{(1-42)}$, INNOTEST® PHOSPHO-TAU$_{(181P)}$, and INNOTEST® hTau Ag, respectively, Fujirebio, Ghent, Belgium). Commercially available single analyte ELISAs were used to measure the concentration of NFL

(NF-light® ELISA, IBL international, Hamburg, Germany), Ng (Neurogranin ELISA, EUROIMMUN, Lübeck, Germany), FABP3 (H-FABP, Human, ELISA kit, Hycult Biotech, Uden, Netherlands), YKL40 (YKL-40 EIA MicroVue™, Quidel Corp., San Diego, USA) and VLP1 (Human VILIP-1 human ELISA, Biovendor, Brno, Czech Republic). The intra- and inter-assay CVs were below 16% for all analytes. The samples were randomized and the analyses were done blinded for diagnosis.

Results

Demographic and biomarker characteristics of the baseline diagnostic groups are summarized in Table 3.6. The CO group was significantly younger than the MCI and AD dementia groups. Demographic and biomarker characteristics of the MCI population are presented in Table 3.7. The MCI stable group was significantly younger than the MCI-AD group.

Comparison between baseline and follow-up diagnoses

Figure 5.9 summarizes the biomarker levels of the CO, MCI, and AD groups. The levels of all candidate biomarkers differed significantly across baseline diagnoses. Subsequent group-wise comparisons showed that NFL differed between all three groups, with increasing levels from CO over MCI to AD. Levels of YKL40 and Ng were higher in both MCI and AD compared with CO, while only AD patients had higher levels of VLP1 compared with CO. FABP3 was significantly higher in MCI, but not in AD, compared with CO.

The biomarker levels of the MCI stable, MCI-AD, and MCI-nonAD groups are shown in Figure 5.10. The levels of NFL, VLP1, and Ng differed across the groups. Group-wise comparisons revealed higher levels of NFL in MCI-stable compared with MCI-AD, while no differences between MCI-AD and MCI stable were found for VLP1 and Ng. The levels of Ng and VLP1 were significantly higher in MCI-AD and MCI stable compared with MCI-nonAD.

Prediction of progression to AD dementia

Logistic regression analyses revealed that A+T+ status (P=.0097) and high levels of $pTau_{181}$ (P=.0113), tTau (P=.0096), and NFL (P=.0279) predict progression from MCI

to AD dementia. Consequent cross-validation produced highest accuracy levels for AT status (Figure 5.11).

In A+T+ MCI subjects, none of the biomarkers significantly predicted progression to AD dementia, i.e. there was no added value of the other markers. In the MCI subjects who were not A+T+, only high NFL levels predicted progression to AD dementia (*P*=.0263; Figure 5.11).

Prediction of MMSE decline

Linear regression analyses indicated that low $A\beta_{1\text{-}42}$ (*P*=.0340), and high $pTau_{181}$ (*P*=.0489), tTau (*P*=.0007), and VLP1 (*P*=.0446) levels predict MMSE decline in MCI. Cross-validation consequently showed that tTau has the lowest error to predict MMSE decline (Figure 5.12).

In the A+T+ MCI subjects, only high $pTau_{181}$ predicted MMSE decline (*P*=.0460; normalized RMSE shown in Figure 5.12), while high $pTau_{181}$ (*P*=.0003), tTau (*P*=.0005), and VLP1 (*P*=.0188) levels predicted MMSE decline in MCI subjects who were not A+T+. In this latter group, $pTau_{181}$ rendered the lowest prediction error based on cross-validation (Figure 5.12).

Discussion

The aim of this study was to compare the predictive powers of a panel of well-studied CSF biomarker candidates with the predictive power of the core AD CSF biomarkers $A\beta_{1\text{-}42}$, $pTau_{181}$, and tTau to assess whether they actually have an added value.

First, when comparing baseline diagnoses and follow-up diagnoses, our findings are in line with previous studies that have shown increased levels of the candidate biomarkers in MCI and/or AD compared with normal as well as in progressive MCI compared with stable MCI [128, 246-268].

In contrast, when assessing the powers to predict progression to AD dementia, of all the candidate biomarkers only NFL was predictive of future progression to AD dementia. However, its predictive power was lower than that of the core AD biomarkers and A+T+ status. As such, NFL has no added value to predict progression

to AD dementia in MCI cases with an A+T+ profile. In the heterogeneous MCI group without an A+T+ biomarker profile, however, NFL was the only predictor of progression to AD dementia, demonstrating the added value of NFL in this MCI subpopulation. In such a heterogeneous group this is not surprising, as several studies previously reported that NFL changes are independent of AD pathology and more related to small vessel disease and axonal damage [249, 269-276].

Regarding biomarker association with MMSE decline, VLP1 was a significant predictor in the entire MCI population as well as in the MCI subjects who did not have an A+T+ profile. However, in the total MCI population, tTau was shown to be the best predictor. Given the heterogeneity of this group, it is not surprising that tTau performs better than the other core biomarkers $A\beta_{1\text{-}42}$ and $pTau_{181}$, as tTau is regarded as an unspecific biomarker of neurodegeneration, while $A\beta_{1\text{-}42}$ and $pTau_{181}$ are considered to be more specific to AD pathology [34, 277]. Indeed, in the A+T+ MCI subjects, only $pTau_{181}$ predicted MMSE decline, with a low error rate, which is in line with previous reports pointing to the relationship between neurofibrillary tangles (and its appropriate biomarkers) and cognitive decline in AD [28, 278, 279]. In the MCI subjects without an A+T+ profile, MMSE decline was also best predicted by $pTau_{181}$. The higher error rate is probably due to the heterogeneity of this group. As the error rate of VLP1 was always higher than $pTau_{181}$ and tTau, we did not find an added value for VLP1.

Although this pilot study benefits from strengths like a clinically well-characterized and representative population as well as medium-to-long follow-up of the MCI subjects, it also has several limitations. For one, the relatively small number of MCI subjects did not enable us to investigate the predictive powers of the biomarkers in more specific subgroups, such as A-T-, A-T+, and A+T- separately. However, as it is unclear what pathology is driving the cognitive impairment in these particular subgroups, we found it reasonable to combine these three categories into a heterogeneous non-A+T+ MCI group. Secondly, the large majority of MCI subjects progressed to AD dementia, which corresponds to the population visiting our memory clinic. The very small number of MCI subjects progressing to another disease than AD, however, did not allow us to draw any conclusions regarding progression to MCI-nonAD. Thirdly, as we retrospectively selected our study cohort,

various tests were used in the neuropsychological examinations that were part of the diagnostic work-up, depending on the diagnostic question and of the abilities of the patient. As a result, not all patients underwent the same tests. Only MMSE was common in the neuropsychological test battery of all patients, which limited the measure of cognitive decline to MMSE decline only. It would therefore be interesting to repeat this study using the same full neuropsychological assessment for the entire population. Finally, due to co-linearity of the biomarkers, it was not appropriate to test multi-biomarker models, to assess whether the candidate biomarkers improved the performance of the core AD biomarkers alone. As such, we were only able to test single-biomarker models.

In conclusion, although the candidate biomarkers showed group-wise differences compared with those reported in previous studies, their predictive powers were limited and did not add value to the core AD CSF biomarkers $A\beta_{1\text{-}42}$, $pTau_{181}$, and tTau. The exception is NFL, which has an added value to predict progression to AD dementia of the heterogeneous MCI group without an A+T+ profile. As such, this pilot study has shown that NFL should be further validated in a prospective, longitudinal clinical study with more sensitive and more extensive cognitive tests. In addition, we advocate for future studies to not only address the value of a possible new biomarker in general, but also assess its value in relationship to the established AD biomarkers.

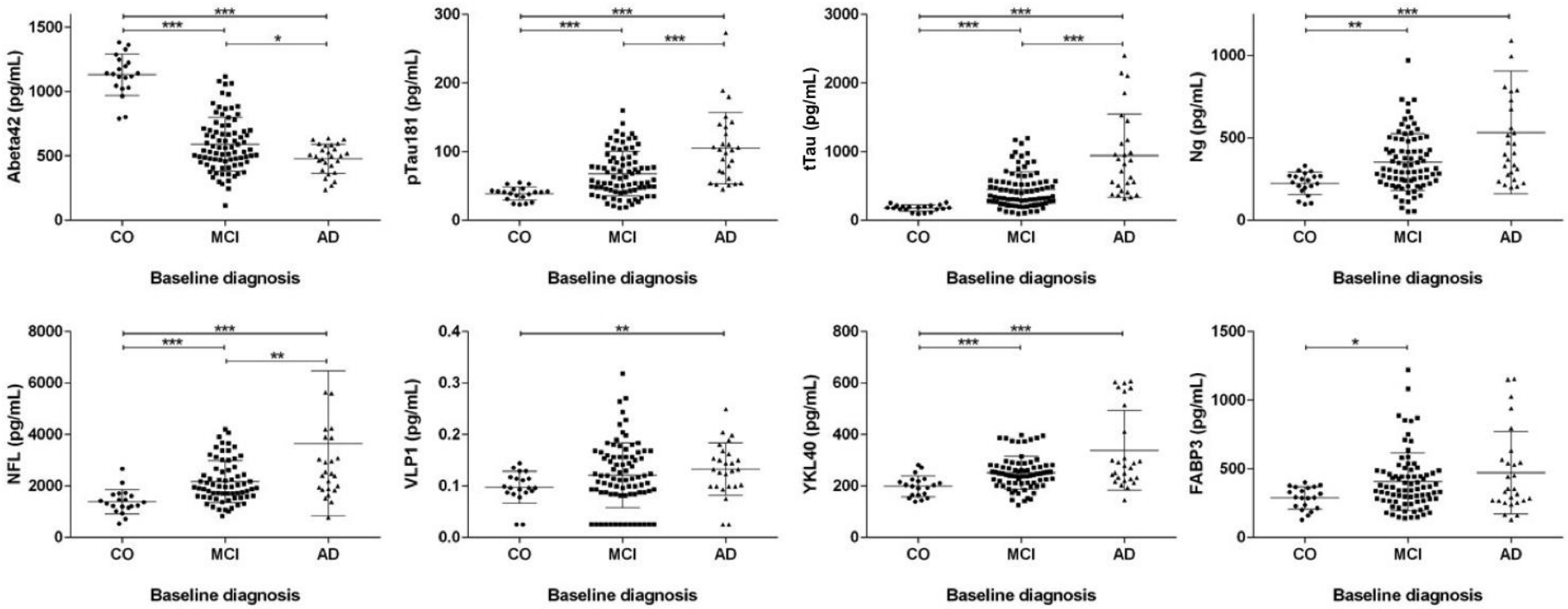

Figure 5.9 Biomarker levels according to baseline diagnosis. *P* value indicators correspond to the values assessed with Mann-Whitney U. Post hoc analysis provided significant differences between groups: * *P*<.0167; ** *P*<.005; *** *P*<.001. Abbreviations: Abeta42, amyloid-β protein of 42 amino-acids; AD, probable Alzheimer's disease dementia; CO, cognitively healthy; FABP3, fatty-acid binding protein 3; MCI, mild cognitive impairment; NFL, neurofilament light; Ng, neurogranin; pTau181, tau protein phosphorylated at threonine 181; tTau, total tau protein; VLP1, visinin-like protein-1.

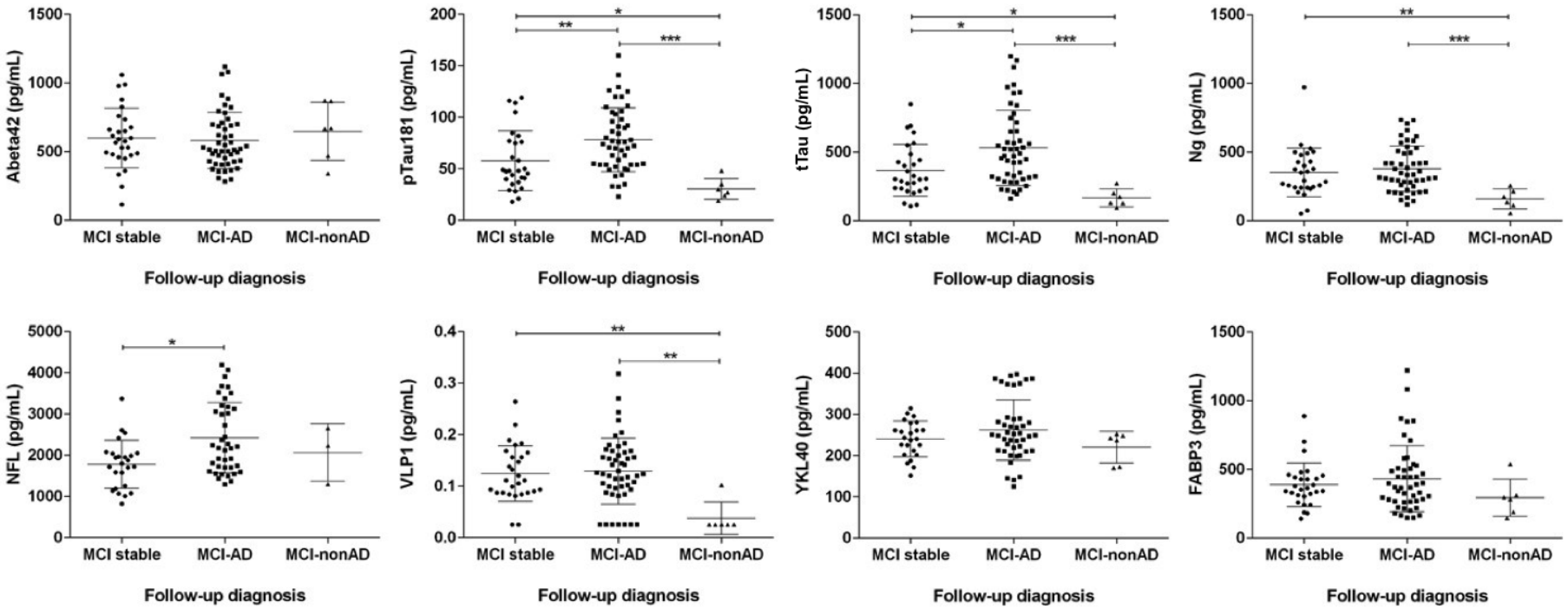

Figure 5.10 Biomarker levels according to follow-up diagnosis. *P* value indicators correspond to the values assessed with Mann-Whitney U. Post hoc analysis provided significant differences between groups: * *P*<.0167; ** *P*<.005; *** *P*<.001. Abbreviations: Abeta42, amyloid-β protein of 42 amino-acids; CO, cognitively healthy; FABP3, fatty-acid binding protein 3; MCI, mild cognitive impairment; MCI-AD, MCI progressing to AD dementia; MCI-nonAD, MCI progressing to dementia other than AD; NFL, neurofilament light; Ng, neurogranin; pTau181, tau protein phosphorylated at threonine 181; tTau, total tau protein; VLP1, visinin-like protein-1.

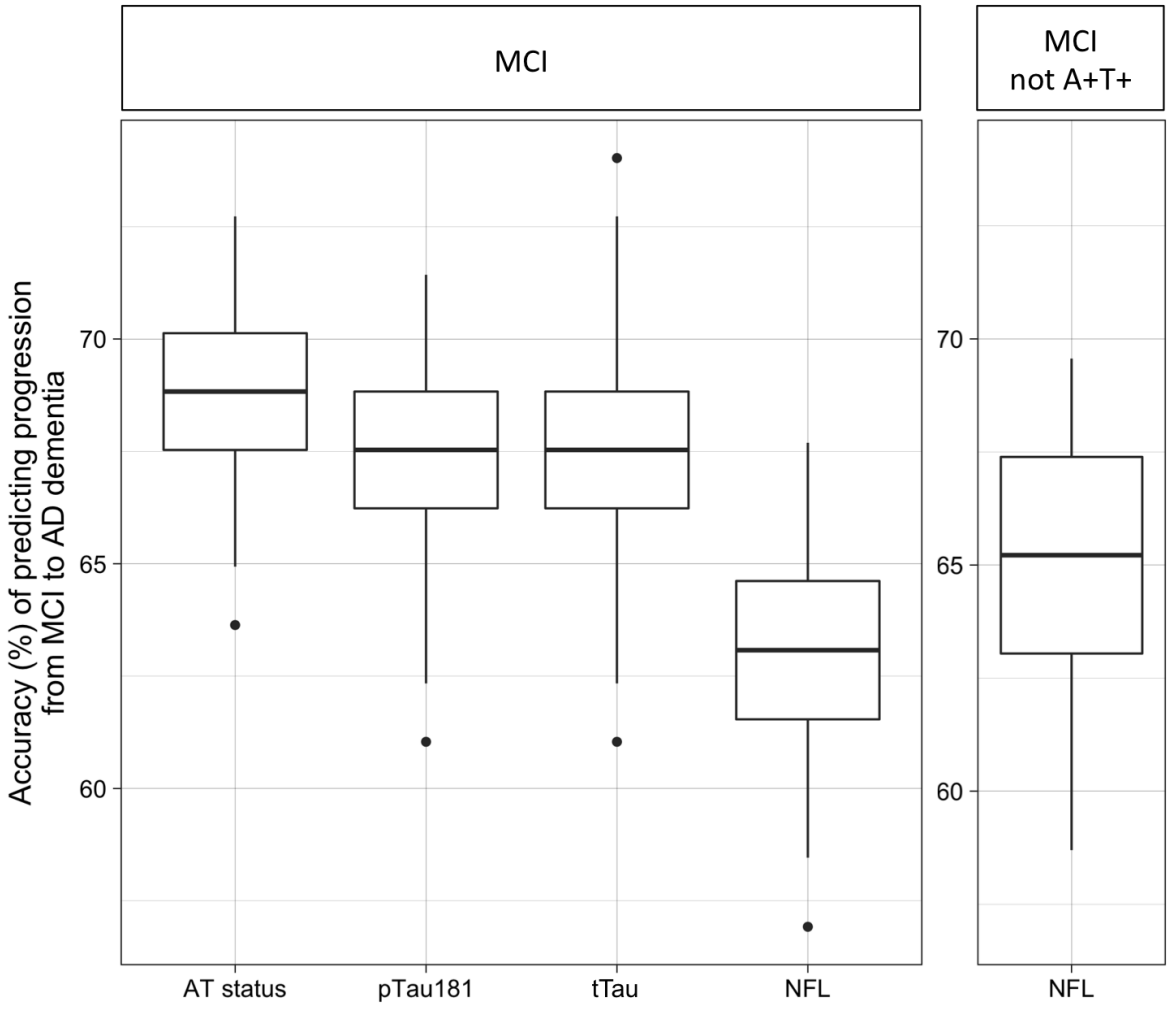

Figure 5.11 Accuracy (%) of predicting progression to AD dementia by baseline biomarker levels of the entire MCI population and those who did not have an A+T+ biomarker profile, calculated by performing 100x 10-fold cross-validation. Abbreviations: AD, Alzheimer's disease; AT status, amyloid and tau status; MCI, mild cognitive impairment; NFL, neurofilament light; pTau181, tau protein phosphorylated at threonine 181; tTau, total tau protein.

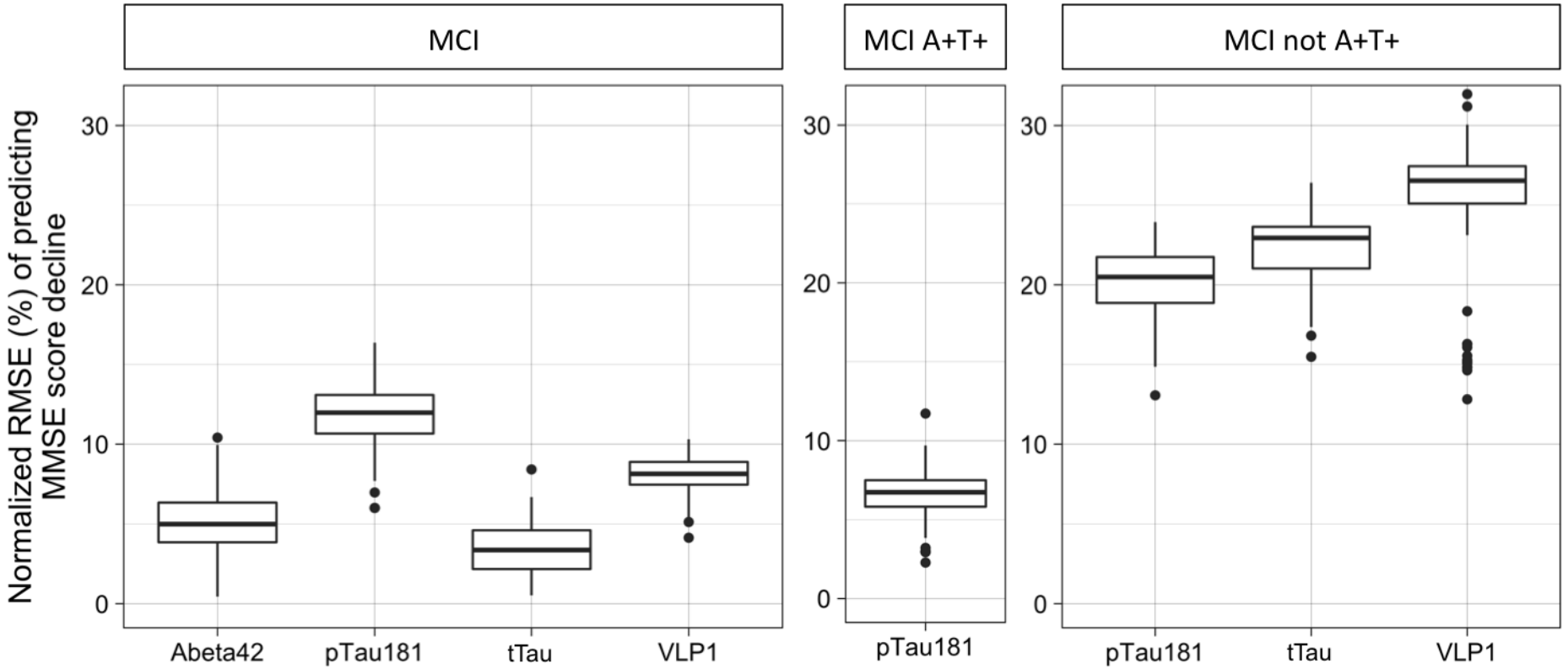

Figure 5.12 RMSE of the prediction of MMSE decline by baseline biomarker levels in the entire MCI population, the A+T+ MCI subjects and in MCI subjects which were not A+T+, based on 100x 10-fold cross validation. RMSE values were normalized for biomarker unit. Abbreviations: Abeta42, amyloid-β protein of 42 amino-acids; MCI, mild cognitive impairment; pTau181, tau protein phosphorylated at threonine 181; RMSE, root-mean-square error; tTau, total tau protein; VLP1, visinin-like protein-1.

Chapter 6. General discussion

This work has focused on biomarkers for an improved classification of AD pathology, both for diagnostic and prognostic purposes. As explained in the introduction, diseases causing dementia have typical but not specific symptoms. As such, the different dementia-causing diseases present with a substantial clinical overlap. Even though no disease-modifying treatments are currently available, accurate differential diagnosis as well as prediction of future decline is of clinical importance for decisions with regard to patient management (treatment, counseling, psychosocial education), and of life decisions (e.g. advanced care planning). Improved diagnostic accuracy would also enhance patient selection for clinical trials, ensuring that the enrolled patients actually carry the targeted pathology. In addition, by improving prognosis, either patients which are likely to progress within the time-frame of the clinical trial could be selected to test drug efficiency on disease progression, or patients who are less likely to progress as they are less advanced and might thus be more responsive to treatment. Once disease-modifying treatments are available, the possibility to predict disease progression will also become important to select patients that could benefit from treatment.

In order to improve the diagnostic and prognostic accuracy, great effort has been put into biomarkers reflecting pathology, enabling clinicians to confirm the presence of pathology while the patient is alive. Currently, only biomarkers for AD pathology are adequately validated for use in clinical practice. As such, the currently established biomarkers for AD have an important value to increase the diagnostic accuracy in case of suspected AD, discriminate AD from other neurodegenerative and cerebrovascular brain disorders, diagnose AD in case of atypical presentations, and identify the early-stage patients that are likely to evolve to AD dementia. However, as explained in the introduction of this PhD thesis, there still is a biomarker overlap between AD and non-AD diseases as well as lack of power to adequately predict time-to-progression in early stages. Moreover, each biomarker category (A/T/N) contains more than one possible measure, while it has been shown that there may be a mismatch between these different measures in the same patient. In this chapter we will discuss the advantages and disadvantages of the established AD biomarkers, elaborate on ways to improve the measures of amyloid,

tau, and neurodegeneration as well as discuss and evaluate new potential candidate biomarkers for AD.

Utility of the biomarkers of the A/T/N classification system in the clinical work-up of Alzheimer's disease

Amyloid plaque pathology

In our study, which was one of the first studies investigating Aβ isoforms in such a diverse selection of clinical diagnoses, we showed that the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio had a higher diagnostic performance than $A\beta_{1-42}$ alone to distinguish between MCI/dementia due to AD and non-AD dementias. For currently unknown reasons, FTLD and VaD also present with decreased levels of CSF $A\beta_{1-42}$, despite the lack of amyloid plaque pathology [60, 272, 274, 280]. As such, CSF $A\beta_{1-42}$ alone has limited specificity as a measure of amyloid plaque pathology. Yet, as CSF $A\beta_{1-40}$ is also decreased in FTLD [102] and VaD [281], while being less affected in AD [166, 282], the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio can be regarded as a better measure of actual amyloid plaque pathology as opposed to CSF $A\beta_{1-42}$ alone [100, 281]. In our study, we indeed found an improvement of the differentiation between AD (both in the dementia and MCI stage) and FTD, not only for $A\beta_{1-40}$, but also for $A\beta_{1-37}$ and $A\beta_{1-38}$.

Regarding the differentiation with VaD, it should be noted that we used clinically diagnosed patients in this study. It has been shown that only 19% of a cohort of clinical VaD patients, who were diagnosed based on structural brain imaging, eventually had a pathological diagnosis of VaD at autopsy. The majority (48%) actually had an autopsy-based diagnosis of AD, while 15% and 19% of them had an autopsy-based diagnosis of DLB and FTLD, respectively [283]. As such, it is possible that our cohort of clinical VaD patients is affected by an overdiagnosis of VaD, which might explain why the use of the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio is not significantly better than CSF $A\beta_{1-42}$ alone. Yet, as the biomarker combinations of $A\beta_{1-42}$ with tTau and $pTau_{181}$ did render (very) high diagnostic accuracy levels, it is not that likely that a large proportion of our clinical VaD cohort actually presents with underlying AD pathology. The same reasoning holds true for differentiation between AD and DLB, where the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio did not significantly improve the discrimination

between the groups. This is probably caused by mixed AD-DLB pathology, which is found in 66-72% of DLB patients [63, 64]. Still, similar to the differentiation between VaD and AD, the combinations of the core AD CSF biomarkers produced good differentiation accuracy levels, making it less likely that the clinical DLB cohort presented with a large proportion of mixed underlying pathologies. However, these are just assumptions and a validation study using a cohort of autopsy-confirmed patients, of whom we know whether amyloid plaque pathology is present, is warranted to fully evaluate the usefulness of Aβ isoforms in mixed pathologies.

As reasoned above, one might thus assume that mixed pathologies or misdiagnoses are the basis for no improved performance of the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio as opposed to the $A\beta_{1-42}$ alone. On the other hand, the ratio did perform significantly better when differentiating the MCI due to AD patients from all non-AD dementias, which again speaks against an overlap caused by mixed pathology or misdiagnosis. A possible mechanism behind this finding could be that CSF $A\beta_{1-42}$ might be more changed in the dementia stage of AD, so that adding information of CSF $A\beta_{1-40}$ will not have a significant impact on the differentiation. In the MCI stage, however, CSF $A\beta_{1-42}$ might be less decreased, in which case the information added by CSF $A\beta_{1-40}$ could indeed have a substantial effect. Indeed, 18 (37%) of the MCI due to AD subjects in our cohort had borderline abnormal or normal levels of CSF $A\beta_{1-42}$, which was the case for only 10 (20%) of the dementia due to AD patients.

Both CSF $A\beta_{1-42}$ and amyloid PET have been shown to be valuable measures of amyloid plaque pathology [53, 284-286]. Although they are regarded as equal measures of amyloid plaques, they still show a mismatch (e.g. CSF+/PET-) in 6-21% of MCI and dementia patients and in 17-21% of cognitively healthy subjects [287-291]. In line with our findings in the abovementioned study, in order to improve the concordance between CSF $A\beta_{1-42}$ and amyloid PET in case of a CSF-/PET+ mismatch, is has been suggested to use ratios of $A\beta_{1-42}$ to other Aβ isoforms, such as $A\beta_{1-40}$ or $A\beta_{1-38}$. Besides improving differential diagnosis, ratios are also reasoned to correct for inter-individual variation in overall Aβ concentrations, as levels of all Aβ isoforms are expected to be increased or decreased in case of an altered production but that only or primarily $A\beta_{1-42}$ will decrease due to amyloid plaque formation [292-294]. As such, in an AD patient with high Aβ production, the levels of $A\beta_{1-42}$

could be in a normal range and lead to a CSF-/PET+ mismatch, while the $A\beta_{1-42}/A\beta_{1-40}$ ratio would be low in such a patient and lead to CSF+/PET+ concordance, as has been shown in a few studies by us and others recently [281, 295-297].

It has been suggested that CSF $A\beta_{1-42}$ can detect amyloid plaque pathology earlier than amyloid PET [298], which could explain some of the CSF+/PET- mismatches, especially at early stages of AD. A possible explanation could originate in the fact that CSF $A\beta_{1-42}$ and amyloid PET measure partly different aspects of amyloidosis. The amyloidosis process starts with the formation of non-fibrillar amyloid species, which results in a decrease of soluble CSF $A\beta_{1-42}$ but cannot be detected by amyloid PET yet [299, 300]. These amyloid non-fibrillar species later become fibrillar and develop into neuritic plaques, which can be detected by amyloid PET [301]. This hypothesis is supported by an autopsy study of a CSF+/PET- case in which many diffuse neocortical amyloid plaques but barely any neuritic plaques were found [302]. In addition, a rare variant of familial AD (the Arctic APP mutation) develops diffuse, and not neuritic, plaques and presents with a CSF+/PET- profile, which is also in line with the abovementioned hypothesis [303].

Methodological issues could of course also be at the basis of mismatches between CSF and PET. For one, errors in pre-analytical and analytical steps in CSF analyses could lead to artificially low levels of CSF $A\beta_{1-42}$ [161, 304-306], while drifts in CSF assays caused by variability in production by the manufacturer could also account for unreliable CSF $A\beta_{1-42}$ levels [307]. However, variability due to the latter issue should be kept under control by the utilization of internal laboratory quality control programs. Anyhow, pre-analytical and analytical issues call for harmonization and standardization of the CSF analytical process, but also the final step of data interpretation needs careful consideration, as explained in the next part of this chapter. In addition, positivity or negativity on CSF and PET measures also depend on the cut-off value. These cut-offs have certain specificity and sensitivity levels and as such also false-positive and false-negative rates that could also cause mismatches between CSF and PET. In an ideal situation, cut-off values are calculated depending on the context of use (e.g. to identify presymptomatic or early MCI due to AD

subjects for clinical trial enrolment, or to confirm AD dementia in a late disease stage).

Efforts to improve measures of amyloid plaque pathology

One of the major difficulties in the CSF field in general is the variability in CSF biomarker analyses resulting from pre-analytical and analytical aspects. Especially CSF $A\beta_{1-42}$ is sensitive to these factors due to its hydrophobic nature and adhesive properties. Pre-analytical factors include the LP and CSF sampling procedures, such as type of collection and/or storage tubes. Analytical aspects include all factors at the assay level, such as variability in assigned calibrator values for different methods, lot-to-lot variability, precision of the method, or inter-operator variability. As a consequence, biomarker levels cannot be compared between laboratories, nor have general laboratory-independent cut-offs been established.

Within the EU Joint Programme Neurodegenerative Disease Research (JPND) consortium 'Biomarkers for Alzheimer's Disease and Parkinson's Disease' (BIOMARKAPD, 2012-2015) much effort has been put into the standardization and harmonization of CSF biomarkers for AD and Parkinson's disease across Europe. Standard operating procedures have been developed on how to collect and store samples [308, 309], how to perform analyses [unpublished results], and how to interpret biomarker results [310, 311]. In addition, in order to compare and possibly pool results from different laboratories, the use of certified reference material has been explored and validated [312, 313] and three reference materials have been certified for calibration of diagnostic assays [314].

Besides improving the measurement of the established CSF Aβ biomarkers, other biomarkers related to Aβ processing are increasingly being explored. One example is BACE1. Results regarding BACE1 in CSF are conflicting, with some studies showing (slightly) increased CSF levels in MCI and AD compared with healthy subjects [128, 131], while others show no significantly altered levels [129, 130]. Our results are in line with the latter, as we also did not find any significant differences or predictive power in MCI.

Another possible new biomarker related to Aβ processing is sAPP. Cleavage of APP by α- and β-secretase leads to the release of sAPPα and sAPPβ, respectively. Both reflect early events of AD pathogenesis [315]. An increase of CSF sAPPα and β in MCI and mild dementia patients with an AD CSF biomarker profile compared with patients without has been shown [171]. Moreover, increased sAPPβ levels in patients with MCI due to AD compared with MCI not due to AD have been reported [170]. However, the results are inconsistent and no significant differences were found for sAPPα and β in a recent meta-analysis when assessed in both dementia and MCI due to AD [77].

It might be hypothesized that increased production of Aβ [315] is associated with increased activity (and levels) of BACE1, which in turn also leads to increased levels of sAPPβ. However, our and other findings in sporadic AD patients speak against this hypothesis, as the levels of BACE1 and sAPPβ are not clearly increased in the CSF of patients with MCI and dementia due to AD. On the other hand, it could be argued that increased production of Aβ takes place at the very beginning of the disease process, decades before clinical disease onset, when plaques first start to accumulate. As such, it may be possible that our study as well as the majority of other studies investigating CSF BACE1 and sAPPβ has been assessing these pathological processes too late in the disease process, and studies in (very) early preclinical stages might be more informative to test whether increased production of Aβ is causing the amyloid plaque pathology in AD. On the other hand, these studies might equally result in negative findings as the mechanisms in sporadic AD are probably different than in familial AD and are rather a deficiency in clearance instead of increased Aβ production [18], which is not expected to affect CSF BACE1 and sAPPβ levels. So far there is no clear evidence of early-stage increased Aβ production, even in familial AD cases that were investigated up to 30 years before expected disease onset [21].

With regard to amyloid PET, one of the earliest and most studied amyloid PET ligands is [^{11}C]Pittsburgh Compound-B ([^{11}C]PiB). At present, [^{11}C]PiB is the most accurate ligand to localize and quantify amyloid depositions [316]. However, the carbon-11 label ([^{11}C]) has a half-life of only 20 minutes, which limits its use to centers with an on-site cyclotron and specialized radiochemistry department. New

generation amyloid ligands are labelled with fluorine-18 ([^{18}F]), which has a half-life of about 110 minutes. [^{18}F]-labelled ligands can thus be produced at the cyclotron site and distributed elsewhere [317, 318]. Four [^{18}F]-labelled ligands are currently being increasingly investigated: [^{18}F]flutametamol [319], [^{18}F]florbetapir [285], [^{18}F]florbetaben [320, 321], and [^{18}F]NAV4694 [322]. All these ligands show binding to Aβ fibrils. However, [^{18}F]flutametamol, [^{18}F]florbetapir, and [^{18}F]florbetaben are less specific than [^{11}C]PiB, as they display white matter binding. As [^{18}F]NAV4694 displays less white matter binding and presents with good pharmacokinetic properties, it is regarded as a potential [^{18}F]-labelled substitute for [^{11}C]PiB [316].

Regarding blood-based amyloid measures, a recent study tested the clinical utility of plasma $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ in two independent cohorts, using immunoprecipitation and mass-spectrometry [323]. They showed that plasma levels of $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ are decreased in A+ as opposed to A- subjects (measured by [^{11}C]PiB) and could adequately differentiate between A+ and A- subjects, ranging from cognitively healthy to dementia. Although the techniques used in this study for Aβ analyses are not useful for high-throughput analyses, the results indicate that plasma Aβ has a clinical value to detect A+ subjects in the entire disease spectrum. In the past, mostly sandwich ELISA methods were used in studies trying to measure plasma Aβ. However, these methods suffered from low sensitivity and produced contradictory results, with unchanged, decreased, or increased levels in patients compared with cognitively healthy controls [324-341]. However, in recent years, ultrasensitive techniques were developed, such as single-molecule array (Simoa) [342] and immunomagnetic reduction (IMR) [343]. Janelidze and colleagues [328] indeed found reduced plasma levels of $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ in preclinical and prodromal AD using the Simoa technique. However, these reductions were small compared with the evident decrease in CSF levels of $A\beta_{1\text{-}42}$. Changes in Aβ are probably detectable in CSF before being discernible in peripheral blood [328], rendering the usefulness of plasma Aβ biomarkers in early diagnosis rather low. In addition, the study by Nakamura and colleagues using IMR [323] compared the diagnostic accuracy of a composite score including plasma $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}40}$, and $APP_{669\text{-}711}$ to CSF $A\beta_{1\text{-}42}$. Similar to the CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}42}$ ratio, one would expect the composite score to perform better than CSF $A\beta_{1\text{-}42}$ alone. Yet, they only found similar diagnostic

accuracies of the plasma composite score compared with CSF $A\beta_{1-42}$. As such, the diagnostic accuracy of plasma compared with CSF amyloid biomarkers is questionable.

Tau pathology

Both CSF $pTau_{181}$ and tau PET have also been shown to be associated with neuropathological findings of neurofibrillary tangles [53] (summarized for different tau PET ligands in [344]). In our study on the value of $pTau_{181}$ for differential dementia diagnosis in an autopsy-confirmed cohort of patients, we were able to show a clear importance of CSF $pTau_{181}$ of the AD CSF biomarker panel to differentiate between AD and non-AD. Especially when differentiating AD from FTLD and CJD, the diagnostic accuracy was high. Due to rapidly progressive neurodegeneration in CJD, CSF tTau levels are generally (very) high in CJD patients [345]. However, as AD patients also sometimes present with similarly high tTau levels, high CSF tTau levels do not perfectly discriminate AD from CJD patients. As CJD does not present with neurofibrillary tangle pathology, which was confirmed by autopsy in our study, CSF $pTau_{181}$ levels are within the normal range in CJD patients. As a result, the ratio $pTau_{181}$/tTau produced a very high diagnostic accuracy to differentiate between AD and CJD patients as it takes both neurofibrillary tangle pathology and neurodegeneration into account.

FTLD is a heterogeneous condition with different underlying neuropathologies, including protein aggregates composed of tau, ubiquitin, and/or TDP-43 [346, 347]. As our study cohort of 17 FTLD patients included only 3 (18%) subjects with underlying tauopathy (FTLD-tau), it might not be surprising that $pTau_{181}$ (in combination with $A\beta_{1-42}$) produced high diagnostic power to distinguish between AD and FTLD. However, as CSF $pTau_{181}$ is not as clearly increased in FTLD-tau as it is in AD [348, 349], the low number of FTLD-tau patients in our cohort is not expected to have led to a significant overestimation of the value of $pTau_{181}$. Still, in order to confirm this statement, our results should be replicated in a larger and independent autopsy-confirmed cohort of FTLD patients with and without underlying tauopathy.

Regarding the differentiation of AD and DLB, we only found low accuracy levels. Retrospectively assessing AD co-pathology in the DLB cohort, we found a probable

explanation for the biomarker overlap, as a large proportion of the autopsy-confirmed DLB subjects, 71% and 42%, showed presence of amyloid plaque and neurofibrillary tangle pathology, respectively, thus possibly representing a mixed AD-DLB neuropathological diagnosis. The accuracy levels were actually lower in the autopsy-confirmed cohort compared with the clinical cohort used to characterize the Aβ isoforms. It is impossible to retrospectively assess how many of the clinical DLB patients used in the Aβ isoforms study presented with mixed pathology, as they did not undergo autopsy. However, as the CSF biomarkers perform a lot better in that cohort, one may assume that the proportion of DLB patients with mixed AD-DLB pathology was lower in that cohort, at least at this (early) disease stage, than in the autopsy-confirmed DLB cohort. As mentioned previously, though, the Aβ isoforms should also be assessed in an autopsy-confirmed cohort in order to evaluate the effect of mixed pathology on the Aβ isoforms levels in CSF. The differentiation between AD and DLB will most probably be improved by biomarkers associated with Lewy bodies, the prominent neuropathological hallmark of DLB, and unrelated to amyloidosis and neurofibrillary tangle pathology.

In the pilot study to identify biomarkers interesting for a prospective validation study, CSF $pTau_{181}$ was one of the best predictors of progression from MCI to AD dementia and of MMSE decline in MCI, especially when used in combination with information on amyloid status. Yet, in our study nor CSF $pTau_{181}$, neither any of the other biomarkers, was able to predict time-to-progression (results not shown). As such, we can merely conclude that CSF $pTau_{181}$ together with amyloid status are the best identifiers of MCI subjects likely to develop AD dementia at a later stage due to underlying AD pathology. The actual prognostic power (i.e. power to predict time-to-progression) remains questionable and other, new biomarkers will probably still outperform CSF $pTau_{181}$ on this specific matter.

Several studies have found a significant, albeit weak to moderate, correlation between CSF $pTau_{181}$ and tau PET. All of these studies used the [^{18}F]AV1451 ligand (also known as [^{18}F]T807 or [^{18}F]Flortaucipir) [350-353]. [^{18}F]AV1451 is the most widely studied tau ligand to date, as it shows good pharmacokinetic properties, high binding affinity and good selectivity for tau over Aβ as well as low white matter binding [43, 354]. As such, its pattern of retention in the cortex is comparable to tau

distribution in AD, while being moderately to strongly associated with disease severity and cognitive decline in AD [42]. However, reliable quantification of [^{18}F]AV1451 retention remains a challenge, due to increasing specific signal in high-binding AD patients during the PET scan as well as off-target binding [355-357]. Results regarding the correspondence between [^{18}F]AV1451 and CSF pTau$_{181}$, and results of [^{18}F]AV1451 in general, should therefore be interpreted with caution.

Besides the half-life of the radioactive tracer, as explained above, PET imaging in general is facing other hurdles as well. Tracers should be able to penetrate the blood-brain barrier, show low toxicity and non-specific binding, have rapid uptake and clearance from the brain, while no radiolabelled metabolites are to enter the brain [358, 359]. Tau PET imaging specifically not only has to comply with these requirements, it also has to tackle different tau isoform compositions, different ultrastructure (paired helical or straight filaments), and varying patterns of tau deposition in different tauopathies [360]. These heterogeneous deposits may not be detected by every tau ligand, or at least not with similar binding affinity. Another challenge is the fact that tau is mainly deposited intracellularly. Hence, the tau tracer should not only be able to cross the blood-brain barrier, but also the cell membrane, leading to specific requirements on molecular size and lipophilicity of the tracer. Last, but not least, current tau tracers show binding affinity for β-sheet formations, which are present not only in tau deposits, but also in amyloid plaques and other misfolded protein deposits. Especially in AD, where tau deposits and amyloid plaques co-localize in neuritic plaques, this is a critical challenge [358, 359].

Although many other tracers have been developed besides [^{18}F]AV1451, none of those can be considered as a reliable imaging measure of NFTs to date, due to all difficulties described above as well as due to specific binding to proteins other than tau. Regarding tau PET, one would thus conclude at present that it is too early to assess its full potential to characterize tau pathology.

Efforts to improve measures of tau pathology

Given the acceptable diagnostic accuracy we found for CSF pTau$_{181}$ in our validation study, we did not further focus on novel biomarkers for neurofibrillary tangles during the remainder of this PhD work. Yet, its diagnostic accuracy to differentiate

AD from VaD and DLB was rather low in our study. In addition, although we found CSF $pTau_{181}$ to be predictive of future progression to AD dementia, it is uncertain whether it can actually predict time to progression. As such, novel biomarkers for the characterization of neurofibrillary tangle pathology for improved differential diagnosis and possibly for prognosis of time-to-progression are still warranted.

An interesting way to address this need might be to target different epitopes and isoforms of tau. Tau is present is six isoforms, resulting from alternative splicing. These isoforms are categorized into two groups, based on their number of microtubule-binding domains: 3 repeats (3R) and 4 repeats (4R) [361, 362]. An approximately equal amount of 3R and 4R tau is present in the healthy adult brain [363], while various tauopathies show changes in the 3R to 4R ratio in their tau deposits. Pick's disease is associated with a predominance of 3R tau, while 4R predominates in corticobasal degeneration, progressive supranuclear palsy, and argyrophilic grain disease, whereas AD still presents with an approximately equal amount or 3R and 4R [364]. Therefore, an interesting approach is the analysis of 3R and 4R tau in CSF. Although this will probably not have added value to detect MCI due to AD, as 3R and 4R are present in approximately equal amounts in AD, it is expected that CSF 3R and 4R tau assays will aid especially in the differential diagnosis of 3R-predominant (e.g. Pick's disease) and 4R-predominant tauopathies (e.g. corticobasal degeneration, progressive supranuclear palsy, argyrophilic grain disease) [365-368]. In addition, as tau tracers with high binding affinity for either 3R or 4R are anticipated in the future, it is expected that CSF analyses of 3R and 4R tau might also yield higher concordance with tau PET than the general $pTau_{181}$ measure.

Regarding different epitopes of tau, an interesting example would be $pTau_{231}$, i.e. tau phosphorylated at threonine 231 [369-371]. High levels of CSF $pTau_{231}$ at baseline have been shown to correlate with cognitive decline and progression from MCI to AD dementia [372]. In addition, levels of CSF $pTau_{231}$ significantly correlate with baseline hippocampal volumes and rates of hippocampal atrophy, independent of disease duration and severity [373]. Most importantly, $pTau_{231}$ has been suggested as a marker of early neurofibrillary pathology. This is of particular interest, because changes in CSF $pTau_{181}$ occur later in the AD disease spectrum than $A\beta_{1\text{-}42}$, meaning that we are currently lacking a very early marker reflecting neurofibrillary

pathology. CSF $pTau_{231}$ might fill this caveat. We performed a small pilot study testing $pTau_{231}$ levels in CSF in patients with MCI and dementia due to AD (i.e. with changes in $A\beta_{1\text{-}42}$ and tTau and/or $pTau_{181}$) and found no differences between the groups for $pTau_{231}$ levels, nor any association to MMSE decline (results not reported). This might be due to the fact that $pTau_{231}$ is already fully changed at the MCI stage. Furthermore, $pTau_{231}$ and $pTau_{181}$ showed a strong correlation (Spearman's rho = 0.88, $P<0.001$), which was, however, lower than the correlation between tTau and $pTau_{181}$ (Spearman's rho = 0.92, $P<0.001$). The correlation of $pTau_{231}$ and tTau was also lower (Spearman's rho = 0.85, $P<0.001$). This might indicate that $pTau_{231}$ and $pTau_{181}$ share a common underlying pathology, but that $pTau_{181}$ is more associated with later stage neurofibrillary pathology that takes place simultaneously with neurodegeneration (measured by tTau). However, this hypothesis still needs to be assessed as well as whether CSF $pTau_{231}$ has an added value in really early MCI subjects as compared to CSF $pTau_{181}$.

In the past, measuring $pTau_{181}$ in plasma was extremely difficult, which resulted in no publications on the subject at all. To the best of our knowledge, only two studies have been recently published reporting results on plasma $pTau_{181}$. The first uses the highly sensitive Simoa technique and found increased levels of plasma $pTau_{181}$ in AD dementia compared with controls. The correlation between plasma and CSF $pTau_{181}$ was weak, though [374]. Unfortunately, this study did not investigate the diagnostic accuracy of plasma as compared to CSF $pTau_{181}$, making it hard to conclude that the plasma marker will perform well enough to replace the CSF counterpart. The second study used IMR to detect $pTau_{181}$ and showed increased levels in MCI due to AD compared with controls and even more increased levels in mild AD dementia patients [375]. However, neither this study reported anything about the relationship with CSF levels, making it hard to evaluate the usefulness of the results.

Neurodegeneration

Atrophy and glucose hypometabolism in AD-related regions are the least specific for AD [34]. Atrophy in AD-related regions is found in a wide variety of neurodegenerative diseases besides AD [67, 68], as well as temporoparietal hypometabolism [69, 70]. As a measure of global, not region-specific

neurodegeneration, increased CSF total tau is also found in traumatic brain injury, stroke, and CJD, correlating with the severity of neuronal damage [158, 376-378]. Due to the unspecific nature of these markers for AD, they are often found to be abnormal in non-AD conditions presenting with normal amyloid biomarkers, a state labelled 'suspected non-AD pathology' (SNAP) [379].

In addition, although the neurodegeneration markers are combined in one category, they each measure somewhat different pathophysiological processes. CSF total tau is a measure of one molecular entity postulating to reflect the intensity of neuronal and axonal degeneration and damage in the brain [97]. [^{18}F]FDG PET is used as a marker for synaptic dysfunction, probably also driven by astrocyte activity [380-382]. Atrophy on MRI not only reflects neuron loss but also other events related to neurodegeneration, such as axonal tracts and dendrites loss, other cell types loss (astrocytes, microglia, etc.), neuroinflammation, and associated changes of interstitial fluid volume [383, 384]. Given these differences in underlying pathophysiological processes, CSF total tau, [^{18}F]FDG PET, and structural MRI, are known to correlate only modestly [385].

As the biomarkers for amyloid pathology and NFTs generally reach abnormality before the onset of clinical symptoms, these markers are considered to be "diagnostic" [36]. In contrast, as neurodegenerative changes occur later in the AD pathophysiology, biomarkers of neurodegeneration are expected to have a better predictive value reflecting clinical progression [36]. However, it remained unclear whether the neurodegeneration markers have similar predictive powers regarding clinical progression. Hence, we felt the need for a head-to-head comparison of the predictive powers of the different neurodegeneration markers and showed that the association between the A, T, and N markers is highly important to predict clinical progression to AD dementia in MCI subjects. Although cortical changes are sensitive to overall clinical progression, the present findings support the coexistence of GM and WM atrophy as a late state of disease pathophysiology with higher specificity for impending cognitive decline in subjects presenting with amyloid and tau abnormalities. It would be interesting to repeat this exercise in a cohort with even longer follow-up and MCI or subjective cognitive decline patients in earlier stages of

the disease, as it might be speculated that [^{18}F]FDG PET might have better predictive powers in less advanced AD patients than the ones we included in our study [21].

Efforts to improve measures of neurodegeneration

In current clinical practice, imaging of neurodegeneration largely depends on visual interpretation of the images. Quantification, however, would enable the detection of small changes and thus improve early diagnosis. In case of MRI, manual segmentation of brain regions-of-interest by neuroanatomical experts is still regarded as the gold standard [386, 387]. This is a very time-consuming procedure with inter-rater variability, which might be lessened by semi-automated techniques. However, such techniques still require a priori information on the region-of-interest, limiting its usefulness for large clinical studies. Fully automated methods have been developed to save both time and costs, and which can be used in larger study cohorts and are easily reproducible [388-392]. Several tools exist, among which: SPM [393], FSL [394], FreeSurfer [395, 396], NeuroQuant [397], BrainVisa [398-400], and ico**brain** (formerly known as MSmetrix) [225, 401-403]. In our validation study of the neurodegeneration biomarkers we used the ico**brain** pipeline, as it has been shown to be a fast, reliable and robust tool to measure brain volume on MRI [225, 401-403]. Examples of automated summary measures of hypometabolism in AD-related regions on [^{18}F]FDG PET are the AD t-sum [404] and the hypometabolic convergence index [244]. Both measures compare individual images with a normative reference dataset in a predefined AD mask.

Next to improvement of the established neurodegeneration markers, many new biomarkers have been postulated to be useful measures of neurodegeneration. Most of these novel approaches attempt to measure a more specific part of neurodegeneration, as opposed to the unspecific measures CSF tTau, glucose hypometabolism, and atrophy.

For example, many studies specifically investigate WM degeneration. A biomarker considered to be a measure of WM changes is CSF NFL, as it is located mainly in large myelinated axons and found to be increased in patients with moderate to severe WM damage [269]. Several studies also reported increased levels of CSF NFL, not only in AD [56, 248, 269, 270, 274, 405-410] but also in a variety of other

neurodegenerative diseases: FTLD [246, 270, 349, 406, 407, 411-416], Parkinson's disease [417], progressive supranuclear palsy [417], (subcortical) VaD [246, 269, 270, 272, 274, 406], amyotrophic lateral sclerosis [349, 405, 409], CJD [408], multiple sclerosis [418], and in CSF and plasma of stroke patients [419, 420]. Indeed, in our study we also found increasing levels of NFL in MCI and AD dementia patients compared with cognitively healthy individuals. Moreover, based on our study there seems to be a particular value of measuring CSF NFL in MCI subjects without changes in both $A\beta_{1-42}$ and $pTau_{181}$ to predict progression to probable AD dementia in our case, but most likely to predict clinical progression in general, given its relationship with a large variety of diseases [246, 269, 270, 272, 274, 349, 405-409, 411-420].

Another useful technique to measure WM integrity is DTI on MRI. DTI, which is sensitive to the Brownian motion of water molecules, enables the measurement of restricted and/or hindered movement of water molecules as they diffuse in the brain. Due to the highly organized nature of the WM, the main diffusion orientation will generally coincide with the orientation of the axons in this tissue. Therefore, DTI can characterize the orientation and integrity of WM fibers [421-424]. It has been shown that the mean diffusivity of the water molecules increases, while the directionality of the water diffusivity (measured by fractional anisotropy) decreases in AD, especially in the temporal and parietal lobes [425-429]. However, although DTI studies provide useful information on WM degeneration in AD and other neurodegenerative diseases, it is an advanced technique with limited applicability in clinical practice.

Another particular field of interest, besides WM changes, is synaptic degeneration, which is postulated to occur before neuronal degeneration. One of the targets in this field is the postsynaptic protein neurogranin. Levels of CSF neurogranin are increased in AD [250-252, 267, 268, 430-435]. In addition, its levels have been shown to predict cognitive decline [251, 252, 268, 436, 437], while seeming to be specific for AD [434]. In line with studies investigating group-wise differences, we also found increased levels in MCI and AD compared with cognitively healthy controls as well as increased levels in MCI subjects progressing to AD dementia compared with those progressing to non-AD. Yet, in contrast to other studies, we did

not find any predictive value for clinical progression for neurogranin. This might be due to the relatively low sample size and the use of MMSE as a measure of cognitive decline.

As mentioned earlier, due to the broad range of possible targets in the field of biomarkers for neurodegeneration, the possibilities seem endless. Here, we only touched upon some of the most relevant ones.

Efforts to improve biomarker-based diagnosis and prognosis in the early stages

One of the major limitations in defining the power of biomarkers to predict future progressing to dementia is the time the MCI patients are followed up. A majority of studies have follow-up periods of one to two years. In such studies it is likely that MCI patients that have not progressed after this short follow-up time are actually at risk of impending progression. This limitation leads to a down-estimation of the prediction accuracies of the biomarkers, or at least limits the accuracy estimate for predicting only fast progression.

Hence, in order to improve the diagnostic and prognostic value of AD biomarkers in the early stages, studies using long follow-up time of preferably more than four years are pivotal. On top of increasing the follow-up time, combining the different AD biomarkers has become another highly promising approach. Combinations can be either within modalities, such as combining different CSF biomarkers, or across modalities, combining CSF biomarkers with MRI for example. Illustrations of such combinations are biomarker profiles [5, 32], classification systems [34], AD-CSF-indices [438-440], ratios of CSF biomarkers [150, 281, 292-295, 297, 441], etc. In addition, novel biomarkers to detect amyloid deposition, neurofibrillary changes, or neurodegeneration are also expected to improve the diagnosis and prognosis of early AD, especially when combined with the current AD biomarkers. Interestingly, also factors that possibly accelerate AD pathology are being investigated increasingly, such as life-style factors, psychiatric symptoms, cerebrovascular burden, risk genes, etc. Such factors will probably be taken into account in the full biomarker-based clinical work-up of dementia diagnosis in the future.

Utility of different biomarker modalities in the clinical work-up of Alzheimer's disease

This PhD thesis described biomarkers of different modalities: CSF, PET, and MRI. However, it is important to not only consider biomarker accuracy but also safety, availability, and economic benefits and drawbacks of each modality when choosing biomarkers for diagnosis, for clinical trial selection, or for research purposes.

CSF is located in the ventricles and is in direct contact with the interstitial fluid that surrounds the brain and extracellular space. In this way, CSF is considered to be a 'window to the brain' as it reflects the biochemical metabolism of the brain, allowing the detection of abnormal protein depositions. CSF is collected through LP, a safe and well-tolerated procedure, especially if performed according to recently published guidelines [309, 442]. Certain contra-indications should be taken into account, such as space-occupying lesions with mass effect in the brain, increased intracranial pressure due to increased CSF pressure, anticoagulant medications, etc. [309]. An LP has a low risk of complications, i.e. back pain and headache, which are both easy to treat and never result in long-term disability [442].

The major advantage of imaging biomarkers is the topographical information they provide, as opposed to CSF biomarkers that are only overall measures of brain functioning. Nevertheless, all chosen CSF biomarkers can be captured with just one LP, also leaving open the possibility of analyzing future biomarkers on the same CSF sample, as opposed to PET imaging that provides information of only one target.

A huge drawback of imaging biomarkers in general is the need of a specialized infrastructure, including specialized personnel for data acquisition and interpretation of the data, large and expensive scanners, safety procedures regarding radiation or strong magnetic fields, and proximity of a cyclotron in case of PET, for instance. As a result, imaging biomarkers are expensive techniques with limited accessibility. In contrast, any trained MD can perform an LP in every hospital or clinic, while the analyses of CSF proteins are far less expensive than imaging.

MRI is less expensive than PET imaging, although still more expensive than an LP. However, as it easily detects structural brain lesions able to cause dementia-like

symptoms, MRI is considered a first-line tool in the diagnostic work-up for AD to exclude other diseases [10, 443].

Although imaging is non-invasive, there are also contra-indications to consider for safety purposes. For PET imaging, the main contra-indication is pregnancy, which is generally not an issue in dementia research. As MR imaging uses strong magnetic fields, patients with pacemakers, defibrillators or other implanted electronic devices, metallic foreign bodies (metal sliver) in their eyes, or an aneurysm clip in their brain cannot have an MRI scan.

Finally, a more practical approach to measure biomarkers as opposed to both CSF and imaging would be blood-based biomarkers. However, the development of blood-based biomarkers is particularly challenging, due to various biological and technical issues. Regarding biological issues, first, a biomarker originating from the central nervous system should cross the blood-brain barrier and when in the blood, its concentrations will be even lower than in CSF due to dilution in the larger blood volume. Second, a biomarker that is also produced in the periphery and is thus not specific for the central nervous system might potentially be undetectable due to high biological background from peripheral sources. Third, the biomarker has to be detected in a matrix of other highly abundant biomolecules (e.g. albumin and immunoglobins), which in addition could interfere with the biomarker itself. Fourth, antibodies possibly present in blood could interfere in sandwich immunoassays. Finally, the biomarker might undergo proteolytic degradation in plasma and be metabolized, and may thus not be detectable.

With regard to technical difficulties, the main issue is sensitivity and specificity of the antibodies used in the assay. Antibodies could cross-react with other proteins and as such result in a measured signal even if the biomarker of interest is absent. This is especially challenging in blood, due to the very dense protein content as opposed to CSF. Lately, ultrasensitive technologies have been developed, which are making near-future blood-based biomarkers more likely [444]. At present, however, blood-based biomarkers are not fully validated yet to replace CSF (and imaging) biomarkers, as was touched upon in the previous sections.

In conclusion, the choice of which biomarker modality to use not only depends on the diagnostic, clinical trial or research aim but also strongly depends on whether a patient presents with contra-indications for a given modality and financial considerations. MR imaging is a first-line tool for AD diagnosis, which is generally reimbursed by health insurances, to exclude other diseases and may at the same time be used to detect contra-indications for LP. In case an MRI cannot be performed due to contra-indications, a CT scan is usually a preferred substitute. As an LP is relatively inexpensive, easy to perform, and allows for the measurement of all the relevant proteins of A, T, and N at once, it is generally preferred over amyloid PET. In patients with contra-indications for LP, however, an amyloid PET scan can aid in the diagnosis of AD.

Chapter 7. Conclusion

This PhD thesis aimed at improving the classification of AD pathology by the characterization and validation of biomarkers. First, improved classification of amyloid (A) was tested through Aβ isoforms and BACE1 in CSF. Using Aβ isoforms was shown to increase diagnostic performance of $A\beta_{1\text{-}42}$ alone and as such these isoforms are valuable additions to the A category. BACE1, however, did not show added value in our pilot study.

The validation of CSF $pTau_{181}$ in an autopsy-confirmed cohort as a measure of NFTs (T) confirmed that $pTau_{181}$ is an essential component of the AD CSF biomarker panel as it showed the highest diagnostic performance to discriminate between AD and non-AD, either alone or in combination with $A\beta_{1\text{-}42}$ or tTau. Regarding the neurodegeneration (N) biomarkers we found that the coexistence of GM and WM atrophy is predictive of impending clinical decline in subjects presenting with amyloid and tau abnormalities. Our pilot study on additional CSF biomarkers to improve the measurement of neurodegeneration included biomarkers that had all been shown to be predictive of cognitive decline in AD in previous studies (NFL, Ng, VLP1, YKL40, and FABP3). Although we were able to confirm some of these findings, we were not able to find any added value of these biomarkers to the core AD CSF biomarkers $A\beta_{1\text{-}42}$, $pTau_{181}$, and tTau. The exception was NFL, which had an added value to predict progression to AD dementia of the heterogeneous MCI group without an A+T+ profile.

Based on the findings reported in this PhD thesis and taking the state-of-the-art regarding other biomarker modalities into account, we first conclude that amyloid can be measured equally well with CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ as amyloid PET, with an advantage for CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ as it measures early non-fibrillar amyloid as well. Second, CSF $pTau_{181}$ is a good measure of NFTs, with tau PET not yet being a valid substitute. As such, we are convinced that it is more prudent to perform one investigation, namely an LP, on a patient to capture measures of A and T (and N) in the same clinical test. As a consequence CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ and $pTau_{181}$ might thus be preferred over amyloid and tau PET. Third, prediction of fast progression from MCI due to AD (i.e. those who are A+T+) to dementia is best predicted by GM and WM volume. Both are measured on MRI, which is already a first-line tool in the diagnostic work-up of AD. Due to the fast and automated measure of GM and WM

volume by ico**brain**, they can be easily implemented in clinical practice. However, whether GM and WM atrophy will also be the best predictors of progression in earlier stages (i.e. early MCI, subjective cognitive decline, and preclinical AD) remains to be tested. Finally, in order to predict progression in a heterogeneous population of non-A+T+ MCI subjects, CSF NFL seems a useful addition to the biomarker panel. The added value of other novel CSF biomarkers still remains unclear and this question should be elucidated first before they can be considered valuable additions to the A/T/N biomarker classification system.

In conclusion, in the current diagnostic work-up of Alzheimer's disease performing MRI and LP to measure CSF $A\beta_{1-42}/A\beta_{1-40}$, $pTau_{181}$, and NFL, and GM and WM volume on MRI will currently give a clinician the means to identify AD pathology and predict whether an MCI patient is likely to progress to AD dementia, as summarized as a step-wise approach in Figure 7.1.

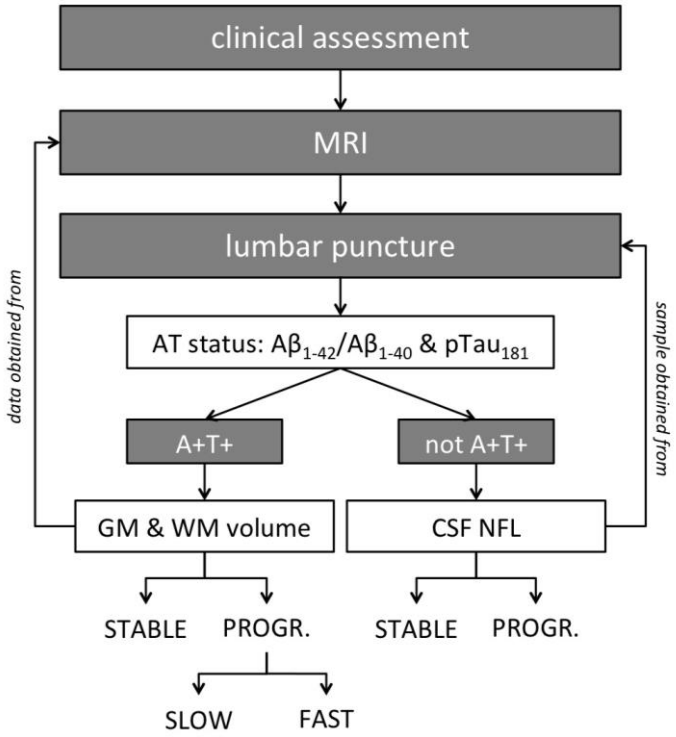

Figure 7.1 Step-wise approach to assess progression to dementia of MCI subjects. By performing an MRI and lumbar puncture, AT status could be assessed by measuring $A\beta_{1-42}/A\beta_{1-40}$ and $pTau_{181}$ in CSF. Next, in A+T+ subjects grey and white matter volume might help to estimate progression to dementia within 2 and 4 years. In subjects who are not A+T+, analyzing neurofilament light in CSF might help to estimate overall progression to dementia. Abbreviations: AT status, amyloid and tau status; CSF, cerebrospinal fluid; GM, grey matter; MRI, magnetic resonance imaging; NFL, neurofilament light; progr.; progression to dementia; WM, white matter.

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Full-length papers in PubMed-cited international peer-reviewed journals

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Dissertations

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