Cognitive profiles in Alzheimer's disease

Recognizing its many faces

Annelies Vonk Noordegraaf - van der Vlies

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Cover: Mosaic of Alois Alzheimer, created using AndreaMosaic: www.andreaplanet.com/andreamosaic. Photos used by courtesy of Matt Hollingsworth, www.openphoto.net, www.photorack.net, www.cepolina.com, and www.morguefile.com.

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ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. F.A. van der Duyn Schouten,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
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De Boelelaan 1105

door

Anne Elisabeth van der Vlies

geboren te Kapelle

In loving memory of my father, D.L. van der Vlies

We're sitting in the opera house;
We're waiting for the curtain to arise
With wonders for our eyes;
We're feeling pretty gay,
And well we may,
"O, Jimmy, look!" I say,
"The band is tuning up
And soon will start to play."
We whistle and we hum,
Beat time with the drum.

We're sitting in the opera house;
We're waiting for the curtain to arise
With wonders for our eyes,
A feeling of expectancy,
A certain kind of ecstasy,
Expectancy and ecstasy... Sh's's's.
Curtain!

Memories - A: very pleasant
Charles Edward Ives

All quotes from classical music literature used in this thesis can be found in the following Spotify Playlist:
http://open.spotify.com/user/anneliesvandervlies/playlist/01gW1kYSspuso11RgeGogz

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From the street a strain on my ear doth fall,
A tune as threadbare as that "old red shawl,"
It is tattered, it is torn,
It shows signs of being worn,
It's the tune my Uncle hummed from early morn,
'T was a common little thing and kind 'a sweet,
But 't was sad and seemed to slow up both his feet;
I can see him shuffling down
To the barn or to the town,
A humming.

Memories - B: rather sad
Charles Edward Ives

1

INTRODUCTION

| DEMENTIA

The world's population is ageing. Improvements in health care in the past century have contributed to people living longer and healthier lives. However, this has also resulted in an increase in the number of people with dementia. Although dementia mainly affects older people, it is not a normal, nor inevitable, part of ageing. Dementia is a syndrome of a chronic and progressive nature, caused by a number of brain disorders that affect - amongst others - memory, language, thinking, behaviour and the ability to perform everyday activities. The total number of people with dementia worldwide has been estimated at 35.6 million in 2010 and is projected to nearly double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050.[1] The number of new cases of dementia each year worldwide is nearly 7.7 million, implying one new case every four seconds. Dementia is diagnosed when there are cognitive or behavioural symptoms that:[2]

1. interfere with daily activities,
2. represent a decline from previous levels of functioning,
3. are not explained by delirium or major psychiatric disorder,
4. are reported by the patient and/or a knowledgeable informant and are objectified by cognitive assessment, and
5. represent cognitive or behavioural impairment in at least two domains.

| COGNITION

The Oxford dictionary defines cognition as "the mental action or process of acquiring knowledge and understanding through thought, experience, and the senses".[3] Largely, cognitive processes take place in the brain, making it difficult to measure or quantify them, although many imaging techniques are available that visualize brain structure and function. The study of the relationship between behaviour, emotion, and cognition on the one hand, and brain function on the other, is what encompasses the field of neuropsychology. In neuropsychology, cognition and behaviour are measured using tests, often administered by paper and pencil. A variety of cognitive domains can be distinguished. Although there is debate about the number and nature of different cognitive domains, in the field of dementia the following domains are generally accepted:

• *Memory:* the ability to acquire and remember new information. Symptoms of memory impairment may be misplacing personal belongings, repetitive conversations and forgetting appointments.

• *Executive functions:* planning, regulating and organising of complex tasks in unstructured situations. Symptoms of impaired executive functions include problems with attention, the inability to manage finances, cook a meal or make decisions.

• *Visuospatial abilities:* the ability to perceive and process visual information and the spatial relationships of objects. Problems with orientation in place, navigating or learning a route, awareness of where objects are in a space, perceiving common objects or faces, or orienting clothing to the body may be expressions of visuospatial impairment.

- *Language:* The systematic, conventional use of sounds, signs or written symbols for communication and self-expression. Symptoms of impaired language functions include problems finding words and speech, spelling, and writing errors.
- *Personality and behaviour:* neuropsychiatric symptoms include depression, apathy, agitation, anxiety, compulsive or obsessive behaviours, disinhibition, and sleep and eating disorders.

The neuropsychological test battery used at the VUmc Alzheimercenter is designed to screen the major cognitive functions and includes the following tests:

- *Mini-Mental State Examination* (MMSE)[4], used to measure dementia severity. The MMSE is a brief 30-point questionnaire test that is used to screen for cognitive impairment. It is commonly used to screen for dementia and to follow the course of cognitive change in an individual over time. In 5 to 10 minutes it samples functions including orientation, memory, language and attention.
- The forward condition of *Digit Span* is used to assess attention.[5] Working memory is assessed with the backward condition of this test. In both cases an extended version with 3 trials per sequence length is used. In the forward condition patients are asked to repeat a sequence of digits. The test starts with a 2 digit sequence and, if the patient repeats enough sequences correctly, may end with 8 digits (score 0-21). In the backward condition, patients are asked to repeat the sequences in reverse order (score 0-21).
- For memory, amongst others the *Visual Association Test* (VAT)[6] is used. Patients are shown cue cards that depict an object and association cards with the previously seen object plus an interacting object and are asked to name the objects. Subsequently, the cue cards are shown without delay and patients are asked to recall the now missing interacting objects (score 0-12).
- *VAT picture naming,* in which patients have to name 12 objects, is used as a measure for language (score 0-12).
- *Category Fluency* is used to assess language function and executive functions. Patients have to produce as many animals as possible in a time frame of 60 seconds.
- *Trail Making Test* (TMT)[7]. The simple part A provides a measure of mental speed. It requires the connection by pencil of numbers (1–25) positioned randomly on a sheet of paper. The more complex part B requires patients to alternate between numbers and letters (e.g., 1–A–2–B–3–C–…), and is used to evaluate executive functioning. For both conditions, the time required for completion is recorded.

Figures 1-4 show some examples of cognitive impairments in Alzheimer's Disease (AD) patients as objectified by neuropsychological tests.
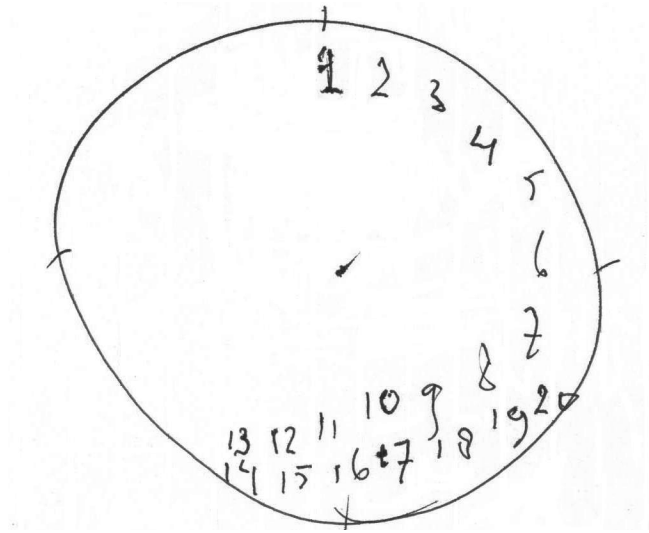
Figure 1. Clock drawing test. Instruction: "draw the face of a clock with all the numbers in it." The drawing indicates problems with planning (unable to plan where the digits have to go in the circle), visuoconstruction (unable to transfer the mental image of a clock to a drawing on paper), visuospatial attention (neglect of the left side of the drawing), and/or inhibition (unable to stop at number 12).
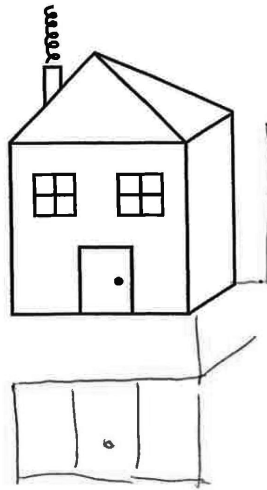
Figure 2. CAMCOG, subtest visiuoconstructional praxis. Instruction: "copy this figure as accurately as possible." Patient is unable to copy the figure, which indicates visuoconstructional impairment.

Zin *thuis*

Figure 3. MMSE. Instruction: "Please write a full sentence." The patient writes the word "*home*" and is unable to produce a full sentence, indicating possible language impairment.

Einde
⑬   ⑩
⑧   ⑨   Ⓘ   Ⓓ
    Ⓑ   ④
    ③
    Begin
⑦   ①
Ⓗ   Ⓒ   ⑤
⑫
Ⓖ
    Ⓐ   Ⓙ
Ⓛ   ②   ⑥
Ⓔ
    Ⓕ   ⑪
Ⓚ
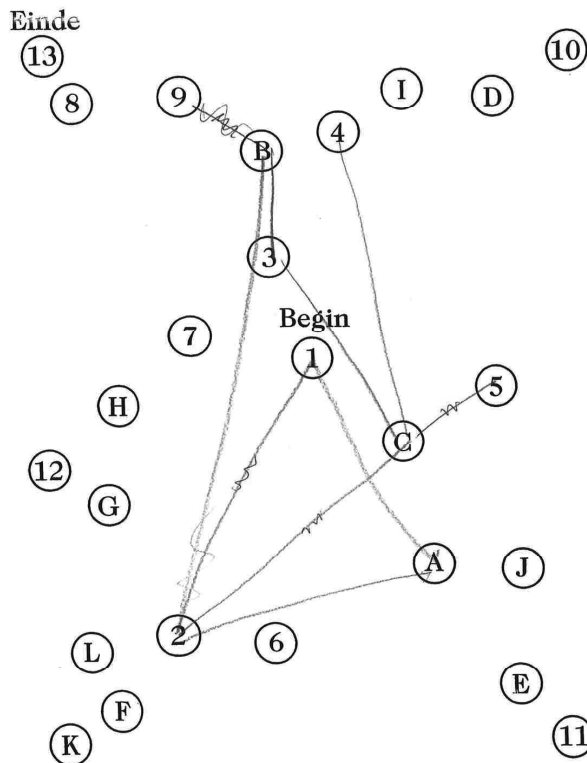
Figure 4. Trail Making Test part B. Instruction: "Start at "1" and connect the circles in consecutive order, alternating between numbers and letters, So 1-A-2-B etc.". The patient makes several types of errors, as he is unable to switch between numbers and letters (going from 1 to 2, skipping the A) and shows inhibition (drawing a line to then nearest object, e.g. going from B to 9).

| ALZHEIMER'S DISEASE

AD is the most common type of dementia. According to the NIA-AA diagnostic criteria[2], dementia is probably caused by AD when:

1. the patient meets the criteria for dementia as described above,
2. there is a clear-cut history of worsening of cognition either by report or by observation,
3. the initial and most prominent cognitive deficits encompass either:
   a. amnestic presentation, including impairment in learning and recall of recently learned information, or
   b. nonamnestic presentation with deficits in language, visuospatial and/or executive function, and
4. there is no evidence of prominent features of a different type of dementia, another concurrent, active neurological disease, or a non-neurological medical comorbidity or use of medication that could have a substantial effect on cognition.

Age at onset

Although dementia mainly affects older people, it is estimated that 10% of the dementia patients is younger than 65 years old. At 34%, the proportion of dementia cases due to AD in patients with early onset dementia is lower than in patients with late onset dementia, in which prevalence has been estimated to be 54%.[8;9] Less than 5% of all AD cases consists of patients with autosomal dominant familial AD caused by mutations in single genes. These patients typically develop AD before the age of 65 years, usually in the 40s or early 50s. However, it should be noted that the majority of early onset AD patients have no known autosomal mutation.

APOE genotype

Genetic factors seem to influence not only familial, but also non-familial cases of AD. The apolipoprotein E (APOE) gene presents in three allelic forms (ε2, ε3 and ε4). The APOE ε4 gene is the most important genetic risk factor for AD. Furthermore, the APOE ε4 genotype lowers the age at onset of AD,[10] although this seems to be the case specifically in late onset AD patients and not in early onset AD patients.[11] Even though the APOE ε4 genotype is a risk factor for AD, many AD patients do not carry the APOE ε4 gene. The question is whether the APOE ε4 genotype also influences the clinical presentation of AD patients once the disease has manifested.

CSF biomarkers

Neuropathologically, AD is characterized by two types of abnormalities, the accumulation of amyloid beta (Aβ) in senile plaques and (hyperphosphorylated) tau in neurofibrillary tangles. Cerebrospinal fluid (CSF) provides a 'reflection of the brain', since it is in direct contact with the brain where the pathological processes causing AD take place. In the CSF, the proteins $A\beta_{1-42}$, tau and p-tau-181 can be measured, as markers for amyloid ($A\beta_{1-42}$) and tangle (tau and p-tau) pathology respectively.[12] These proteins are generally accepted as diagnostic biomarkers for AD with sensitivity around 80-90% and specificity around 90-95% against normal aging.[12] However, the concentrations of these CSF biomarkers vary greatly even among AD patients. The clinical impact of this variation has thus far scarcely been understood.

MRI features

It is thought that the accumulation of Aβ triggers cell death, and hence cerebral atrophy. Cerebral atrophy in AD, visualized using Magnetic Resonance Imaging (MRI), usually starts in the medial temporal lobe, extends to the remainder of the cortex and eventually results in global atrophy.[13-16] Furthermore, patients with AD often have some degree of small vessel disease. White matter hyperintensities (WMH), which are thought to reflect microvascular pathology, are more prevalent in AD patients than in healthy elderly. Finally, in recent years microbleeds -small rounded regions as seen on specific MRI sequences, which are thought to represent blood breakdown products- have also been associated with AD. It would seem plausible that specific brain damage is related to specific symptoms, but –at least in AD- this often does not seem to be the case. Also with relation to MRI features there is great variation between AD patients, and much research regarding the clinical impact of this variation remains to be done.

Cognition in AD

Amongst AD patients, there is substantial variability with regard to cognitive profiles. Although in the majority of AD patients memory impairment is the most salient cognitive feature, some patients present with atypical symptoms. In these patients other cognitive domains, such as language, visuospatial abilities and/ or executive functions, are more severely impaired than memory. Some of the most common forms of atypical AD are Posterior Cortical Atrophy (PCA), logopenic variant primary progressive aphasia (PPA) and frontal AD. In PCA, patients present with visual impairment in the absence of significant primary ocular disease to explain the symptoms and relatively spared memory and language abilities.[17] Structural imaging shows focal or asymmetric atrophy in parietal and/or occipital regions with relative sparing of the medial temporal lobe. Logopenic variant PPA is characterized by prolonged word-finding pauses, and impaired auditory verbal short-term memory, while at least in the initial stages of the disease, episodic memory is relatively spared. Although the PPA syndrome can also be found in other types of dementia, emerging evidence suggests that logopenic variant PPA is underpinned by AD pathology in at least two-thirds of the cases.[18] The frontal AD phenotype is formed by a subgroup of AD patients presenting chiefly with impairments of behaviour and executive functions. Overall, these atypical variants of AD are more likely than typical AD to present in younger age groups.[18] However, in clinical practice we find that patients that do not fulfil the criteria for these well-defined subtypes still show substantial variability in the degree to which the various cognitive functions are impaired, regardless of dementia severity.

Current issues

Little is known about the biological factors underpinning the spectrum of cognitive impairment in AD patients. Furthermore, patients with atypical presentations often go misdiagnosed for years before they are finally diagnosed with AD. A deeper understanding of the different AD subtypes, their pathologies and factors that drive the differentiation of phenotype is therefore important to obtain a wider acknowledgement of the many

faces of AD. Additionally, many endeavours are being undertaken to develop therapeutic interventions that slow down or even bring a halt to the progression of the disease. Consideration of subtypes of AD is likely to be important in the development and allotment of (future) tailor-made therapies.

By definition, patients with AD show progression. Median survival with AD is estimated at 7 years,[19] but there is much individual variability around these median estimates. Overall, patients show a mean cognitive decline of 2 MMSE-points per year, but there is large variation in rate of cognitive decline between patients. In the interest of both the patient and his/her support system - e.g. enabling them to make plans for the future and attempt to maintain autonomy for as long as possible- and the success of therapeutic interventions, it is important to be able to identify which patients are likely to show fast progression and which patients will deteriorate more slowly.

| AIMS

The overall objective of this thesis was to obtain insight in cognitive profiles and rates of cognitive decline in AD and to explore which factors are involved in driving these different phenotypes. The thesis focuses on two aspects of clinical manifestation:
• Profiles of neuropsychological impairment in AD
• Rate of cognitive decline in AD

We aimed to explore the extent to which APOE genotype, CSF biomarkers and MRI findings explain differences in cognitive profiles and rate of cognitive decline. All studies were performed in AD patients only, in order to explore different subgroups within the same disease.

Profiles of neuropsychological impairment in Alzheimer's disease

In **chapter 2.1** we investigated whether APOE ε4 carriership results in different cognitive profiles in AD patients. In **chapter 2.2**, $A\beta_{1-42}$, tau and p-tau-181 levels as measured in CSF were used to identify clusters of CSF biomarkers and relate these to differences in neuropsychological profiles. **Chapter 2.3** covers the relationship between global atrophy and WMH as seen on MRI and neuropsychological impairment.

Rate of cognitive decline in Alzheimer's disease

In **chapter 3.1** the effect of age at onset on the rate of cognitive decline in AD patients was examined in a longitudinal study. In addition, the possibly modifying influence of APOE genotype on this association was studied. In **chapter 3.2** we studied the influence of CSF biomarkers $A\beta_{1-42}$, tau and p-tau-181 on the rate of cognitive decline. Finally, the influence of cerebral microbleeds on the rate of cognitive decline was studied in **chapter 3.3**.

In **chapter 4** the main findings of this thesis are summarized and discussed and recommendations for future research are given.

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Zu meiner Zeit, zu meiner Zeit	In my time, in my time
ward Pflicht und Ordnung nicht entweiht.	duties and order were not profaned.
Der Mann ward, wie es sich gebühret,	The husband was, as is proper,
Von einer lieben Frau regieret,	governed by a beloved wife,
Trotz seiner stolzen Männlichkeit.	despite of his proud manliness.
O gute Zeit, o gute Zeit!	O good old days, o good old days!
Die Fromme herrschte nur gelinder,	The pious wife just ruled more mildly,
Uns blieb der Hut und ihm die Kinder;	we had our hats and he had the children;
Das war die Mode weit und breit.	this used to be a widespread custom.
O gute Zeit, o gute Zeit!	O good old days, o good old days!

Die Alte
Wolgang Amadeus Mozart

2

PROFILES OF NEUROPSYCHOLOGICAL IMPAIRMENT IN ALZHEIMER'S DISEASE

2.1

COGNITIVE IMPAIRMENT IN AD IS MODIFIED BY APOE GENOTYPE

A.E. van der Vlies[1], Y.A.L. Pijnenburg[1], T. Koene[2], M. Klein[2],
A. Kok[3], P. Scheltens[1], W.M. van der Flier[1]

[1] Alzheimer Center & Department of Neurology, Neuroscience Campus Amsterdam,
[2] Medical Psychology and [3] Clinical Chemistry, VU University Medical Centre,
Amsterdam, the Netherlands.

| ABSTRACT

2.1

Aim

We examined whether impairment in specific cognitive domains in AD differed according to APOE genotype and age at onset.

Methods

Cognitive functions of 229 consecutive AD patients were assessed using Visual Association Test (VAT), Memory Impairment Screen+ (MIS+), VAT object naming, fluency test and Trail Making Test (TMT). Dementia severity was assessed using MMSE. ANOVA's were performed with APOE genotype and age at onset as independent variables and sex, education and MMSE as covariates.

Results

28% of patients were APOE ε4 negative, 58% heterozygous and 14% homozygous. A significant association between APOE genotype and VAT and MIS+ was found when correcting for sex and education. An interaction effect between APOE genotype and age at onset on VAT and VAT object naming was found, with young carriers performing worse than young non-carriers. By contrast, when additionally correcting for MMSE, a significant association between APOE genotype and VAT object naming, TMT-A and TMT-B was found, with non-carriers performing worse than carriers.

Conclusion

Memory was more impaired among APOE ε4 carriers than among non-carriers. By contrast, naming, executive functions and mental speed were more impaired among APOE ε4 non-carriers. This suggests that the APOE genotype modifies the clinical phenotype in terms of cognitive impairment in AD.

| INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, characterized by gradually increasing cognitive impairment.[1] Typically, loss of memory is followed by global cognitive decline. However, in some patients impairment in other cognitive functions, such as visuospatial skills, language and executive functions, is more salient than memory impairment.[2] It has been suggested that patients with a young age at onset present with a different cognitive profile compared with patients with late onset[3-5], although other studies find no major differences between these two groups.[6;7]

The apolipoprotein E gene (APOE) is an important risk factor for AD.[8-11] Among the three major isoforms of APOE, the most common allele is ε3, followed by ε4 and ε2.[12] The ε4 allele increases the risk of AD and has been associated with an earlier age at onset.[8;10;13;14] Furthermore, the presence of APOE ε4 has been associated with more severe memory impairment, while it has been suggested that non-memory deficits may be more prominent in the absence of APOE ε4.[6;15-17] This effect may be more prominent among patients with early-onset.[16]

The aim of this study was to assess whether cognitive impairment differed according to APOE genotype in patients with AD. We hypothesized that APOE ε4 would be associated with a more prominent memory impairment, whereas the absence of the APOE ε4 allele would show a more severe impairment of non-memory functions. Secondly, we investigated whether age at onset, independently or related to APOE genotype, influenced patterns of cognitive impairment.

| METHODS

Patients

We consecutively recruited 229 patients with AD from the outpatient memory clinic of the Alzheimer Center of the VU University Medical Centre (VUmc). Standardized dementia assessment included medical history, informant-based history, physical and neurological exam, laboratory tests, neuropsychological testing, electroencephalogram (EEG) and magnetic resonance imaging (MRI) of the brain. Diagnoses of probable AD were made in a multidisciplinary consensus meeting according to the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) diagnostic criteria.[1] Age at onset was estimated by subtracting the duration of the cognitive complaints as reported by the patient and/or caregiver from the age at the time of neuropsychological testing. Age at onset of 65 years or younger was considered as early-onset. The level of education was classified using the system of Verhage,[18] ranging from 1 (low) to 7 (high). The study was approved by the local Medical Ethical Committee. All patients gave written informed consent.

Neuropsychological assessment

The neuropsychological test battery was designed to screen the major cognitive functions and included the following tests. Dementia severity was assessed using the *Mini-Mental*

State Examination (MMSE).[19] For memory, the *Visual Association Test* (VAT)[20] was used. Patients are shown cue cards with an object and association cards with the previously seen object plus an interacting object and are asked to name them. Subsequently, the cue cards are shown without delay and patients are asked to recall the now missing interacting objects (score 0-12). Furthermore, the delayed recall of the *Memory Impairment Screen+* (MIS+)[21] was used. Patients are presented with a sheet of paper with items belonging to different categories, and are asked to memorize all items. After approximately 3 minutes, patients are asked to recall the items. Category cues are presented if necessary. The number of items retrieved is recorded (0-12). *VAT object naming* was used as a measure for language (0-12). Language functions were additionally assessed using *Fluency*, where patients have to produce as many animals as possible in a time limit of 60 seconds. Furthermore, the *Trail Making Test* (TMT)[22] was used. The simple part A provides a measure of mental speed. It requires the connection by pencil of numbers (1–25) positioned randomly on a sheet of paper. The more complex part B requires patients to alternate between numbers and letters (e.g., 1–A–2–B–3–C–…), and was used to evaluate executive functioning. For both conditions, the time required for completion is recorded. Depending on the specific test, data were available for 123-215 patients.

APOE

DNA was isolated from 10 ml EDTA blood. APOE genotype was determined at the Department of Clinical Chemistry of the VUmc with the Light Cycler APOE mutation detection method (Roche Diagnostics GmbH, Mannheim, Germany). APOE was analysed according to number of APOE ε4 alleles.

Statistical analysis

Since the neuropsychological scores were not normally distributed, all were log transformed. Group comparisons were performed using χ^2-tests, Kruskal-Wallis tests or analysis of variance (ANOVA) when appropriate. Two-way ANOVA's with APOE ε4 dose (0, 1 or 2) and age at onset ($\leq$ 65 or > 65) as independent variables and neuropsychological scores as dependent variables were performed. In the first model sex and education were entered as covariates. In the second model, dementia severity -measured using the MMSE- was additionally corrected for.

| RESULTS

Sixty-five (28%) patients were APOE ε4 negative, 132 (58%) heterozygous and 32 (14%) homozygous. No association between APOE status and age at onset, sex, level of education or dementia severity as assessed using the MMSE (table 1) was found. Furthermore, 92 (40%) patients had an early disease-onset while 137 (60%) had a late onset. Age groups did not differ with respect to APOE status, sex, level of education or MMSE. A t-test showed that there were no differences in disease severity between age groups (MMSE young: m(sd)= 20(6); old: m(sd)= 20 (5), t= -0.141, p= 0.888).

Table 1. Demographic and clinical characteristics by APOE ε4 dose.

	0 ε4	1 ε4	2 ε4
N	65	132	32
Sex (% female)	33 (51%)	74 (56%)	21 (65%)
Age at onset	67 (9)	66 (9)	66 (9)
Age at onset ≤ 65 years	27 (42%)	52 (39%)	13 (41%)
Level of education*	5 (1-7)	5 (1-7)	5 (2-7)
MMSE[a]	21 (5)	20 (6)	20 (7)

Data are represented as mean (standard deviation), median (range) or n (percentage).
MMSE = Mini Mental State Examination
* education using Verhage's classification (range 1-7)[18]
[a] data available for 196 patients

Two-way ANOVA's were performed to assess the effects of APOE and age at onset on neuropsychological functioning (table 2). In the first model, sex and level of education were entered as covariates.

Table 2. Neuropsychological measures by APOE ε4 dose and age at onset.

	age at onset	0 ε4	1 ε4	2 ε4	Model 1	Model 2
VAT	≤ 65 years	5.9 (4.3)	4.2 (3.5)	1.6 (2.0)	A, B	
	> 65 years	4.9 (3.7)	3.9 (3.6)	2.9 (2.7)		
MIS+	≤ 65 years	2.9 (2.9)	3.3 (2.9)	1.0 (1.4)	A	A
	> 65 years	3.1 (2.7)	2.7 (2.7)	0.8 (1.1)		
VAT object naming	≤ 65 years	11.2 (1.0)	11.3 (1.8)	10.6 (3.3)	B	A
	> 65 years	10.5 (2.4)	11.2 (1.2)	11.7 (0.5)		
Fluency	≤ 65 years	11.7 (3.9)	12.6 (5.2)	14.4 (4.4)		
	> 65 years	12.1 (4.6)	12.5 (5.2)	11.5 (4.2)		
TMT-A[†]	≤ 65 years	105 (53)	124 (149)	69 (44)	A	
	> 65 years	112 (111)	91 (63)	122 (82)		
TMT-B[†]	≤ 65 years	359 (240)	221 (135)	162 (60)	A	
	> 65 years	265 (114)	250 (116)	282 (172)		

Data are represented as mean (standard deviation). Please note that raw neuropsychological scores are shown, while statistical analyses were performed with log transformed scores. Numbers of data available for analyses ranged from 215-123 patients.
VAT = Visual Association Test, MIS+ = delayed recall of the Memory Impairment Screen+, TMT = Trail Making Test.
Model 1: two-way ANOVA with APOE genotype and age at onset as independent variables and sex and level of education as covariates. Model 2: two-way ANOVA with APOE genotype and age at onset as independent variables and sex, level of education and MMSE-score as covariates.
[†]: lower scores signify better (i.e. faster) performance, A: significant APOE effect with $p < 0.05$, B: significant interaction effect between APOE and age at onset with $p < 0.05$.

A main effect of APOE was observed for both memory tests (VAT: $F_{2, 207}$ = 3.14; p= 0.045; MIS+: $F_{2, 149}$ = 4.16; p= 0.017), with APOE ε4 carriers performing worse than non-carriers. There was no main effect of age at onset, but a significant interaction term was observed for VAT ($F_{2, 207}$ = 3.60; p= 0.029), implying that the effect of APOE ε4 was more pronounced in patients with early-onset AD. For VAT object naming, an interaction between APOE and age at onset was observed ($F_{2, 197}$ = 3.82; p= 0.023), although main effects were not significant (figure 1). Older patients performed better, while younger patients performed worse as their number of APOE ε4 alleles increased. There was no effect of either APOE or age at onset on fluency or the TMT.

In the second model, we corrected for dementia severity using MMSE as an additional covariate. The effect of APOE on memory as measured using VAT lost statistical significance, but the effect on the MIS+ remained ($F_{2, 123}$ = 3.99; p= 0.021) with homozygous ε4 carriers performing worse than heterozygous or non-carriers (figure 2). By contrast, APOE ε4 carriers performed better than non-carriers on VAT object naming ($F_{2, 167}$ = 3.27; p= 0.041). Neither the main effect of age at onset, nor the interaction term was significant. There was no effect of APOE or age at onset on fluency. However, a main effect of APOE on both the simple and complex condition of the TMT (TMT A: $F_{2, 156}$ = 4.92; p= 0.008; TMT B: $F_{2, 99}$ = 6.77; p= 0.002) was found, with APOE ε4 carriers performing better than non-carriers. Neither the effect of age at onset, nor the interaction terms were significant.
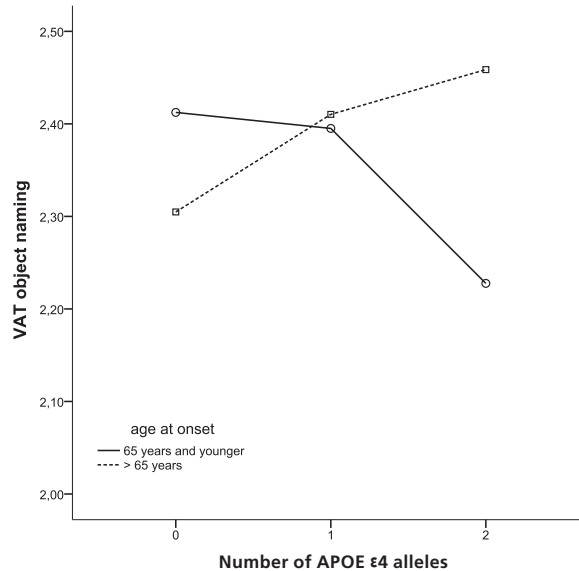
Figure 1. Effect of number of APOE ε4 alleles and age at onset on Visual Association Test object naming. X-axis shows groups classified according to number of APOE ε4 alleles, Y-axis shows estimated mean scores after log transformation and correction for sex and level of education (model 1). Two-way ANOVA revealed no main effect of APOE or age at onset. A significant interaction effect between APOE and age at onset ($F_{2, 197}$ = 3.82; p= 0.023) was found. Older patients performed better, while younger patients performed worse as their number of APOE ε4 alleles increased.
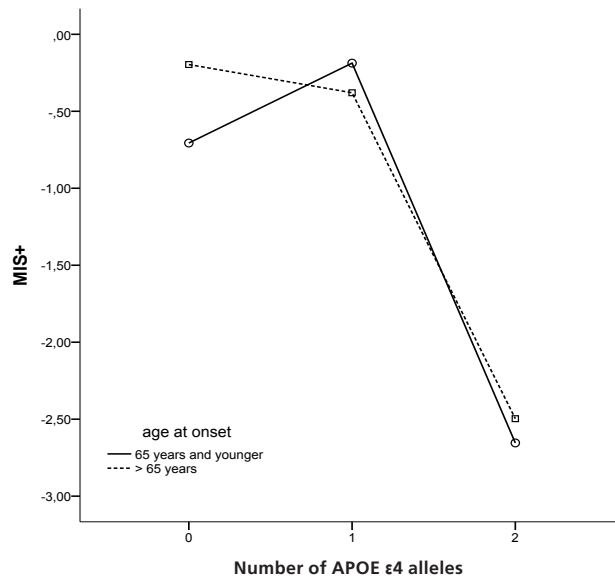
Figure 2. Effect of number of APOE ε4 alleles and age at onset on the delayed recall of the Memory Impairment Screen+. X-axis shows groups classified according to number of APOE ε4 alleles, Y-axis shows estimated mean scores after log transformation and correction for sex, level of education and MMSE-score (model 2). Two-way ANOVA revealed a main effect of APOE genotype ($F_{2, 123}$ = 3.99; p= 0.021); APOE ε4 carriers performed worse than non-carriers. No main effect of age at onset and no interaction effect was found.

| DISCUSSION

We found that APOE ε4 positive patients with AD perform worse on memory-related tasks than APOE ε4 negative patients, while in contrast, APOE ε4 positive patients perform better than APOE ε4 negative patients on neuropsychological tests of naming, mental speed and executive functions. Although we observed no direct effect of age at onset on cognitive performance, the effects of APOE genotype were partly modified by age at onset.

Our findings are consistent with previous studies reporting relatively more severe memory impairment in APOE ε4 positive patients.[6;16] Moreover, we extend on these findings as, by contrast, we found naming, executive functions and mental speed to be more severely impaired in non-carriers than in carriers, suggesting a different cognitive phenotype.

On visual inspection, homozygous APOE ε4 patients performed worse on memory tests than heterozygous and APOE ε4 negative patients. Several studies have reported hippocampal volume loss in APOE ε4 carriers.[23-25] If the APOE ε4 genotype affects the hippocampus, this is a likely explanation for the more severe memory impairment we found in APOE ε4 positive patients. By contrast, we found that non-carriers had worse naming abilities than carriers. Additionally, non-carriers showed more impairment of the executive functions and mental speed than heterozygous and homozygous APOE ε4 patients. Possibly, APOE non-ε4

genotype affects other regions of the brain, such as the cingulate gyrus and parietal lobes, with relative sparing of medial temporal structures.[2;26]

2.1

Several studies have reported that the presence of the APOE ε4 allele influences the age at onset in a dose-effect related way. In the present study this association was not observed, as there was no difference in age at onset between groups. There are several possible explanations for this result. One of these is the way the age at onset was estimated. It is likely that the disease-process in people developing AD starts before they or their caregivers start complaining about a change in one or more cognitive functions. Therefore, subtracting the duration of the cognitive complaints from the age at the time of neuropsychological testing might not reflect the real age at onset. Secondly, the specific referral pattern may explain the lack of association between APOE and age at onset in our study. The memory clinic from which patients have been recruited is a tertial referral center with specific focus on the diagnosis of early onset AD, resulting in a high proportion of patients with early onset (40%). Because of this specific focus, it is likely that, besides young APOE ε4 carriers, a lot of young AD patients with non-APOE ε4 genotype were recruited. The referral pattern as described above might in part explain the lack of association between APOE genotype and age at onset.

No main effect for age at onset on any of the neuropsychological tests was found. However, in accordance with a previous study[16], our findings suggest that the effect of APOE genotype on cognitive phenotype is partly modified by age at onset for memory and naming abilities.

One of the strengths of our study is the relatively large sample size. Furthermore, by using neuropsychological tests we were able to evaluate separate cognitive functions in a quantitative way. A possible limitation is the lack of a cognitive test specifically designed to assess praxis and visuospatial functions, as it has been suggested that these cognitive functions may be relatively more often impaired in patients with early onset and in APOE ε4 negative patients.[2;15;17] Moreover, since the neuropsychological test battery was not completely standardized, not every test was administered to every patient.

The presence or absence of the APOE ε4 allele in AD patients may be associated with a steeper cognitive decline. Several studies have been performed to investigate this relationship. However, their results are far from unequivocal, reporting slower[27;28], faster[29] and the same[12] rate of cognitive decline in APOE ε4 carriers compared tot non-carriers. Although the cross-sectional design of the present study did not allow us to assess the possible effect of APOE genotype on cognitive decline over time, it is conceivable that our results are clouded by the fact that some patients were more severely demented than others. To account for this effect, we corrected for dementia severity as measured by the MMSE. After this correction, we found a stronger association between APOE and executive functions and mental speed. Our results suggest that comparably or even more severely affected APOE ε4 positive patients still perform better on tests of mental speed and executive functions. Further study is necessary to investigate the influence of APOE ε4 genotype on the rate of decline of specific cognitive domains.

| ACKNOWLEDGEMENTS

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2.1

2.2

CSF BIOMARKERS IN RELATIONSHIP TO COGNITIVE PROFILES IN ALZHEIMER'S DISEASE

A.E. van der Vlies[1], N.A. Verwey[1,2], F.H. Bouwman[1], M.A. Blankenstein[2], M. Klein[3], P. Scheltens[1], W.M. van der Flier[1]

[1] Alzheimer Center & Department of Neurology, Neuroscience Campus Amsterdam, [2] Clinical Chemistry and [3] Medical Psychology, VU University Medical Center, Amsterdam, the Netherlands.

| ABSTRACT

Objective

To investigate the relationship between CSF biomarkers and cognitive profiles in AD.

Methods

We included 177 AD patients. Digit Span, Visual Association Test (VAT), VAT object naming, Trail Making Test (TMT) and category fluency were used to asses cognitive functions. Disease severity was assessed using MMSE, functional impairment was rated by CDR. In CSF, levels of Amyloid-Beta 1-42 ($A\beta_{1-42}$), tau and tau phosphorylated at threonine 181 (p-tau) were measured. K-means cluster analysis was performed with the three biomarkers to obtain 3 clusters. MANOVA for repeated measures was performed with CSF-cluster as between subjects factor, neuropsychological z-scores as within subjects variable and age, sex and education as covariates.

Results

Cluster 1 consisted of 88 patients (49%) with relatively high levels of $A\beta_{1-42}$ and low levels of tau and p-tau. Cluster 2 contained 72 patients (41%) with relatively low levels of $A\beta_{1-42}$ and high levels of tau and p-tau. Cluster 3 was made up of 17 patients (10%) with low levels of $A\beta_{1-42}$ and very high levels of tau and p-tau. No differences between clusters on age, sex, education, APOE genotype, disease duration, functional impairment or disease severity were found. Patients in cluster 3 performed worse on VAT, TMT-A and B and fluency.

Conclusions

Clusters of CSF biomarker levels are related to cognitive profiles in AD. A subgroup of patients with extremely high CSF levels of tau and p-tau shows a distinct cognitive profile with more severe impairment of memory, mental speed and executive functions, which cannot be explained by disease severity.

| INTRODUCTION

Memory is generally considered to be the primary cognitive function affected by Alzheimer's disease (AD).[1;2] However, it is increasingly recognized that the clinical presentation of AD is heterogeneous, with other cognitive functions such as language, praxis, executive and visuospatial skills sometimes showing more prominent impairment than memory.[2-4] Factors influencing cognitive phenotype in AD are largely unknown.

Cognitive impairment in AD is caused by neuropathological changes in the brain, specifically the formation of senile plaques, containing the Amyloid-beta protein, and neurofibrillary tangles, containing hyperphosporylated tau.[5] In the cerebrospinal fluid (CSF), levels of Amyloid-Beta 1-42 ($A\beta_{1-42}$), total tau and tau phosphorylated at threonine 181 (p-tau) can be measured. These proteins are increasingly accepted as diagnostic biomarkers for AD with sensitivity around 80-90% and specificity figures of 90-95% against normal aging.[6] However, even among AD patients large variation in the levels of these biomarkers exists. A previous study used cluster analysis to identify subgroups of AD based on CSF biomarkers.[7] Although each subgroup presented with specific clinical characteristics, it is unclear whether the subgroups showed different cognitive profiles.

Little has been done to study the relationship between CSF biomarkers and cognition. Some studies have found an association between CSF $A\beta_{1-42}$ levels and disease severity in AD as measured by the MMSE.[8;9] Others did not find such a relationship, but reported an association between tau and memory.[10] In this study, we set out to form clusters based on CSF biomarkers $A\beta_{1-42,}$ tau and p-tau, and aimed to investigate their relationship with profiles of cognitive impairment in a cohort of AD patients.

| METHODS

Patients

We recruited 177 consecutive patients with AD and available CSF from our outpatient memory clinic. Standardized dementia assessment included medical history, informant-based history, physical and neurological exam, laboratory tests, neuropsychological testing, electroencephalogram (EEG) and magnetic resonance imaging (MRI) of the brain. Diagnoses of probable AD were made in a multidisciplinary consensus meeting according to the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) diagnostic criteria.[1] Diagnosis was made without awareness of the results of the CSF analysis. Disease duration was defined as the time in years between the first symptoms as reported by the patient or caregiver and the first visit to the memory clinic. Functional impairment was assessed using the *Clinical Dementia Rating* (CDR).[11] Dementia severity was assessed using the *Mini-Mental State Examination* (MMSE).[12] The level of education was classified using the system of Verhage,[13] ranging from 1 (low) to 7 (high). The study was approved by the local Medical Ethical Committee and all patients gave written informed consent.

CSF analysis

CSF was obtained by LP between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 10 ml polypropylene tubes. Within two hours, CSF samples were centrifuged at 1800 g for 10 minutes at 4°C. A small amount of CSF was used for routine analysis, including total cells (leucocytes and erythrocytes), total protein and glucose. CSF was aliquoted in polypropylene tubes of 0.5 or 1 ml and stored at -80°C until further analysis. CSF $A\beta_{1-42}$, tau and p-tau were measured with Innotest sandwich ELISA as described previously.[14] The inter-assay coefficient of variation for $A\beta_{1-42}$ was 12.6%, 12.6% for tau and 10.3% for p-tau. In our lab, the following cut-off values are used: CSF $A\beta_{1-42}$ < 495 pg/ml, CSF tau > 356 pg/ml and p-tau > 54 pg/ml.[15;16] The lumbar puncture (LP) and the collection of clinical data were less than 5 months apart. In the majority of patients (n=96, 54%), all data were collected on the same day.

Neuropsychological tests

The neuropsychological test battery was designed to screen the major cognitive functions and included the following tests. The forward condition of *Digit Span* was used to assess attention.[17] Working memory was assessed with the backward condition of this test. In both cases an extended version with 3 trials per sequence length was used. In the forward condition patients are asked to repeat a sequence of digits. The test starts with a 2 digit sequence and, if the patient repeats all sequences correctly, may end with 8 digits (score 0-21). In the backward condition, patients are asked to repeat the sequences in reverse order (score 0-21). For memory, the *Visual Association Test* (VAT) was used.[18] Patients are shown six cue cards with an object on it, and association cards with the previously seen object plus an interacting object and are asked to name them. Subsequently, the cue cards are shown without delay and patients are asked to recall the now missing interacting objects. This procedure is repeated for a second trial. Scores of the first and second trial are summed to obtain a total score (range 0-12). *VAT object naming* was used as a measure for language (0-12). Furthermore, the *Trail Making Test* (TMT) was used.[19] The simple part A provides a measure of mental speed. It requires the connection by pencil of numbers (1-25) positioned randomly on a sheet of paper. The more complex part B requires patients to alternate between numbers and letters (e.g., 1–A–2–B–3–C–…), and was used to evaluate executive functioning. For both conditions, the time required for completion is recorded. Executive functions and language were additionally assessed using a test of category fluency, in which patients have to produce as many animals as possible in a time limit of 60 seconds.

Statistical analysis

For statistical analysis, SPSS version 12.0 was used. Bivariate Pearson correlations were performed to analyze the relationship between individual CSF biomarkers and neuropsychological tests. To investigate the relationship between combinations of biomarkers and neuropsychological performance, K-means cluster analysis was performed with $A\beta_{1-42}$, tau and p-tau as cluster variables to obtain 3 clusters.[20] This analysis assigns patients to groups, aiming to obtain a maximal difference in cluster variables between clusters and a minimal difference between patients within any given cluster. Group comparisons were

performed using χ^2-tests, Kruskal-Wallis tests or analysis of variance (ANOVA). TMT-A and B scores were log-transformed. All neuropsychological data were transformed into z-scores, to allow direct comparison of test results. On the TMT, a higher score signifies a worse performance, while on all other tests a higher score corresponds with a better performance. Therefore the z-scores on the TMT-A and B were inverted by computing –1 * z-score. MANOVA for repeated measures was performed with CSF-cluster as between subjects factor, neuropsychological test (z-scores of 7 tests) as within subjects variable and age, sex and education as covariates. The significance level was set at $p < 0.05$. Interactions were considered significant if p-values were lower than 0.10.

| RESULTS

There were 177 AD patients. Age (mean ± sd) was 69 ± 8 and 93 patients (53%) were female. Median level of education was 5 (range: 1-7). Patients were mild to moderately demented, with a mean MMSE score of 22 ± 4 and CDR score of 0.9 ± 0.5.

We first performed bivariate Pearson correlations between individual CSF biomarkers and neuropsychological tests. There was a negative correlation between CSF tau levels and VAT (r = -.24; p < .01) and category fluency (r = -.15; p = .05). Furthermore, a negative correlation between CSF p-tau levels and VAT was found (r = -.25; p < .01). No other correlations between CSF biomarker levels and neuropsychological tests were found.
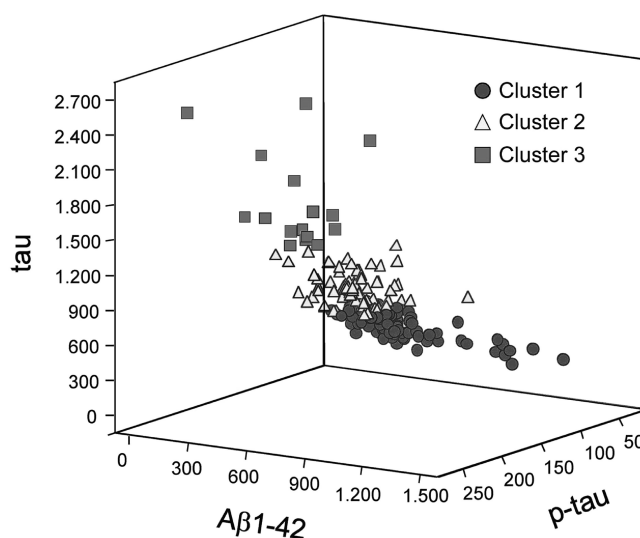
Figure 1. Distribution of CSF Aβ_{1-42}, tau and p-tau levels in pg/ml by cluster. The x-axis shows the Aβ_{1-42} levels, the y-axis the tau-levels and the z-axis the levels of p-tau. Cluster 1 consists of patients with relatively high levels of Aβ_{1-42} and relatively low levels of tau and p-tau. Cluster 2 contained patients with relatively low levels of Aβ_{1-42} and relatively high levels of tau and p-tau. Cluster 3 is made up of patients with low levels of Aβ_{1-42} and very high levels of tau and p-tau.

After categorization using K-means cluster analysis, cluster 1 consisted of 88 patients (49%) with relatively high levels of $A\beta_{1-42}$ and relatively low levels of tau and p-tau (table 1). Cluster 2 contained 72 patients (41%) with relatively low levels of $A\beta_{1-42}$ and relatively high levels of tau and p-tau. Cluster 3 included 17 patients (10%) with low levels of $A\beta_{1-42}$ and very high levels of tau and p-tau. The distribution of $A\beta_{1-42,}$ tau and p-tau over the clusters is shown in figure 1.

Table 1. Demographic and clinical characteristics by CSF cluster.

	Cluster 1	Cluster 2	Cluster 3
N	88 (49%)	72 (41%)	17 (10%)
$A\beta_{1-42}$, pg/ml	530 (235)	448 (103)[#]	425 (122)
Abnormal $A\beta_{1-42}$ level[1]	50 (57%)	50 (69%)	13 (76%)
Tau, pg/ml	446 (112)	844 (154)[†]	1720 (430)[†]*
Abnormal tau level[1]	69 (78%)	72 (100%)[†]	17 (100%)[†]
P-tau, pg/ml	65 (15)	103 (25)[†]	170 (38)[†]*
Abnormal p-tau level[1]	65 (74%)	70 (97%)[†]	17 (100%)[†]
Sex (N, % female)	47 (53%)	37 (51%)	9 (53%)
Age	70 (9)	68 (8)	68 (9)
Level of education[2]	5 (2-7)	5 (2-7)	5 (1-7)
ApoE genotype, ε4 positives[3]	52 (64%)	42 (67%)	12 (75%)
Disease duration (years)	3 (3)	3 (2)	2 (1)
CDR	0.9 (0.5)	0.9 (0.4)	0.8 (0.5)
MMSE	22 (4)	22 (4)	22 (3)
Infarct on MRI	2 (2%)	1 (1%)	0 (0%)
Medical history of:			
– head injury	2 (2%)	1 (1%)	1 (6%)
– diabetes mellitus	3 (3%)	4 (6%)	0 (0%)
– hypertension	12 (14%)	9 (13%)	4 (24%)
– hypercholesterolaemia	6 (7%)	6 (8%)	0 (0%)
– myocardial infarction	6 (7%)	6 (8%)	0 (0%)

Data are represented as mean (SD), median (range) or n (%).
[1] The following cut off values are used in our lab: CSF $A\beta_{1-42}$ < 495 pg/ml, CSF tau > 356 pg/ml and p-tau > 54 pg/ml. [15;16]
[2] Education using Verhage's classification[13]
[3] Data available for 160 patients
MMSE: Mini Mental State Examination
difference with Cluster 1 (p < .05), † difference with Cluster 1 (p < .01), * difference with Cluster 2 (p < .01).

Except for $A\beta_{1-42}$ in cluster 3, all biomarkers showed significant differences between clusters. The proportion of patients with abnormal CSF biomarker levels is shown in table 1. For

Aβ$_{1-42,}$ no difference in the proportion of patients with an abnormal level was found between clusters. The proportion of patients with abnormal levels of tau and p-tau was higher in clusters 2 and 3 than in cluster 1 (p < .01). No differences between clusters on age, sex, education, APOE genotype, disease duration, MMSE, or CDR were found. Furthermore, there were no differences between clusters in the occurrence of infarcts, head injury, or vascular risk factors (table 1).

Table 2. Neuropsychological measures by cluster.

	Cluster 1	Cluster 2	Cluster 3
Digit span forward	10.7 (2.9)	11.2 (2.8)	10.8 (2.8)
Digit span backward	6.8 (2.5)	7.0 (2.8)	5.9 (2.7)
VAT naming	11.5 (1.1)	11.1 (1.5)	11.3 (1.6)
VAT memory	5.2 (3.7)	4.4 (3.3)	2.8 (2.8)
TMT-A †	87 (53)	90 (78)	144 (100)
TMT-B †	331 (169)	314 (166)	401 (158)
Category Fluency	13.3 (4.7)	13.1 (5.2)	11.3 (3.6)

Data are represented as mean (SD). Raw data are shown, whereas for the analyses z-scores were used.
VAT: Visual Association Test, TMT: TrailMaking Test
† seconds; lower scores signify better (i.e. faster) performances

neuropsychological tests
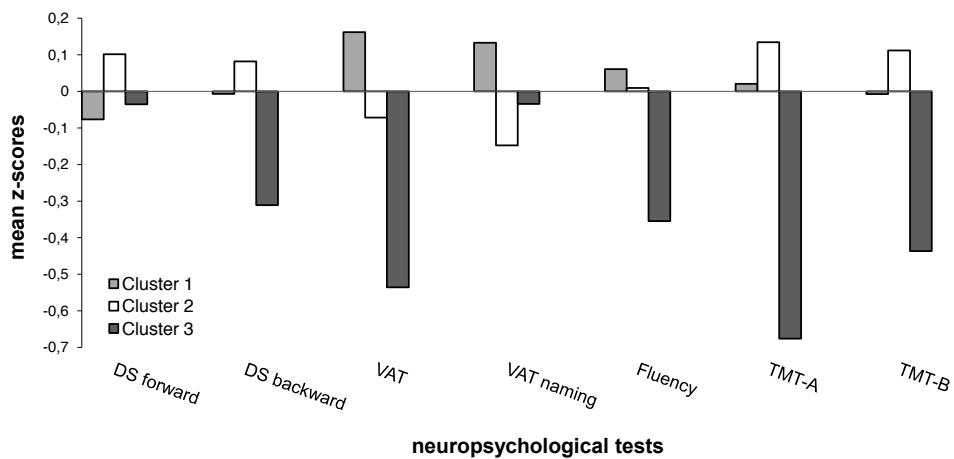
Figure 2. Mean neuropsychological z-scores by cluster. The x-axis shows the individual neuropsychological tests, the y-axis shows the mean z-scores. DS = Digit span; VAT = Visual Association Test, TMT = TrailMaking Test. Note that z-scores of the TMT were inverted, so that on all tests higher z-scores indicate better performances. MANOVA for repeated measures revealed main effects for cluster (F$_{2, 171}$ = 3.29; p= 0.04), neuropsychological test (F$_{5, 817}$ = 3.88; p= 0.002) and an interaction between neuropsychological test and cluster (p= 0.06). Patients in cluster 3 performed worse on VAT, TMT-A and B and category fluency. Patients in cluster 1 performed relatively better on object naming and VAT.

The mean raw scores by cluster for the neuropsychological tests are shown in table 2. After adjustment for age, sex and education, MANOVA for repeated measures revealed main effects for cluster ($F_{2, 171}$ = 3.29; p= 0.04) and neuropsychological test ($F_{5, 817}$ = 3.88; p= 0.002). Furthermore, there was an interaction between neuropsychological test and cluster (p= 0.06), indicating that the profiles of neuropsychological test scores differed among clusters. Figure 2 illustrates that patients in cluster 3 performed worse on the VAT, TMT-A and B and category fluency than patients in cluster 1 and 2. Patients in cluster 1 performed better on object naming and VAT than patients in the other clusters.

| DISCUSSION

The present study shows a difference in cognitive profile between clusters of AD patients based on the combined CSF levels of $A\beta_{1-42}$, tau and p-tau. Patients with low levels of $A\beta_{1-42}$ and extremely high levels of tau and p-tau performed worse on tests of memory, mental speed and executive functions than patients with less abnormal biomarker levels. In contrast, patients with levels of $A\beta_{1-42}$, tau and p-tau close to normal, showed less impairment of naming and memory abilities than patients with more extreme biomarker levels. These differences in cognitive profile could not be explained by a difference in disease duration, functional impairment as measured by CDR, or severity as measured by MMSE.

We used cluster analysis to relate the combination of three CSF biomarkers to cognitive impairment. A previous study used a clustering technique based on the CSF biomarker levels of $A\beta_{1-42}$, tau and ubiquitin.[7] The authors report that the diagnostic accuracy to identify AD subgroups using this clustering technique exceeds the accuracy of using the markers individually or in combination of twos. In our study we used p-tau rather than ubiquitin, since it is a more specific biomarker for AD. In agreement with the former study, we found that cluster analysis is a useful technique to identify meaningful subgroups in AD based on combinations of CSF biomarkers. Moreover, we extend on the earlier findings by showing that the CSF-based subgroups have distinct cognitive profiles.

The few studies of CSF biomarkers and cognition that have been conducted previously, studied individual markers in relationship to a single or global cognitive function. These studies reported an association between high tau levels and memory impairment in AD patients[10], as well as an association between high $A\beta_{1-42}$ levels and naming abilities.[8] In the current study, we found correlations between high tau levels and memory impairment and between and high p-tau levels and memory impairment. However, the association between $A\beta_{1-42}$ levels and naming abilities could not be replicated. In this study we investigated a combination of CSF biomarkers in relationship to a range of cognitive functions in AD patients. We found that patients in cluster 1, with relatively high levels of $A\beta_{1-42}$ and relatively low levels of tau and p-tau, performed better on object naming, while patients in cluster 3, with low levels of $A\beta_{1-42}$ and very high levels of tau and p-tau, showed more impairment of memory, mental speed and executive functions. By combining these biomarkers, we identified groups of patients with specific cognitive profiles which seem to suggest subtypes of AD.

These findings may have important clinical implications. In recent years, more and more effort has been directed towards the development of therapies for AD. The heterogeneous nature of AD is increasingly being recognized, suggesting that it may not be likely that a therapy which applies to all AD patients will be found in the near future. In a future of individualized therapy, it is increasingly important to be able to identify meaningful subtypes. CSF biomarker clusters may provide a way to stratify patients for tailor-made allotment of therapies.

One of the strengths of this study is the relatively large sample size. Also, by using a neuropsychological test battery, rather than a single screening test, we were able to evaluate separate cognitive functions in a quantitative way. A possible limitation of the study is the cross-sectional nature of the study. Longitudinal studies are needed to confirm our findings. Another limitation is the fact that we have no postmortem data to verify the clinical diagnosis of AD. Although we cannot rule out that some of these patients were misdiagnosed as AD, all patients in this cluster met the clinical criteria for probable AD.[1]

The most striking features of the patients in cluster 3 are the high levels of tau and p-tau. Extremely elevated tau levels are typically found in Creutzfeldt-Jacob Disease (CJD). However, the extreme tau-levels in CJD are considerably higher (between 5000 and 10.000 pg/ml) than the levels of the patients in cluster 3.[21] Also in a proportion of patients with frontotemporal dementia (FTD), elevated CSF tau levels are found. But in contrast with AD, p-tau levels are generally normal in FTD.[6] Therefore, we do not think that this offers an explanation for the high tau levels in our study. Furthermore, a previous study has also reported a wide range of tau levels in AD patients, in which the diagnosis was confirmed at autopsy.[22] A third possibility would be that patients in cluster 3 suffered from acute ischemic stroke, since elevated tau levels are found in these patients up to 5 month after the stroke.[23] However, no sign of stroke was seen on the MRI of any of the patients in cluster 3.

It might be hypothesized that the patients with high levels of tau and p-tau have progressed to a more advanced neuropathological stage, in which the neurofibrillary tangles are more widespread, resulting in a more severe memory impairment.[24] The more severe impairment of mental speed and executive functioning in these patients may be the consequence of neurofibrillary tangles in the higher order association cortex, resulting in synaptic malfunction and impaired signaling between nerve cells.[25] However, the fact that all clusters showed similar disease severity as measured by MMSE and similar functional impairment as measured by CDR is in contrast with this hypothesis. Furthermore, a lack of association between CSF levels of Aβ_{1-42}, tau and p-tau and plaque and tangle burden according to Braak's neuropathological stages has been reported in autopsy confirmed AD patients.[26] Alternatively, this subgroup may be a specific subtype that, irrespective of disease stage, presents with high tau levels. CSF tau is generally regarded to be an a-specific marker for neuronal damage and degeneration. It has been suggested that both tau and p-tau reflect the intensity of the disease process in AD, with higher CSF levels of these biomarkers indicating a more aggressive disease and a more rapid progression.[27] Possibly, the patients in

the cluster with very high levels of tau and p-tau will show a more intense disease process. Further longitudinal study is needed to investigate whether subgroups of patients based on their CSF biomarker profiles show differences in rate of decline. Furthermore, since MRI phenotype (e.g. cerebral vascular damage and atrophy) may be the missing link between CSF biomarker profiles and cognitive phenotypes, it is worth studying the relationship between CSF biomarker profiles and patterns of atrophy and white matter hyperintensities in the future.

| ACKNOWLEDGEMENTS

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2.2

2.3

ASSOCIATIONS BETWEEN MRI MEASURES AND NEUROPSYCHOLOGICAL IMPAIRMENT IN EARLY AND LATE ONSET ALZHEIMER'S DISEASE

A.E. van der Vlies[1], S.S. Staekenborg[1], F. Admiraal-Behloul[2], N.D. Prins[1], F. Barkhof[3], H. Vrenken[3,4], J.H.C. Reiber[2], P. Scheltens[1], W.M. van der Flier[1]

[1] Alzheimer Center & Department of Neurology, Neuroscience Campus Amsterdam, [3] Department of Radiology, Image Analysis Center and Alzheimer Center, [4] Department of Physics and Medical Technology, VU University Medical Center, Amsterdam, the Netherlands. [2] Division of Image Processing, Dept of Radiology, Leiden University Medical Center, Leiden, The Netherlands.

| ABSTRACT

Aim

To assess the associations of global atrophy and white matter hyperintensities (WMH) with neuropsychological function in early and late onset Alzheimer's Disease (AD).

Methods

We included 107 patients with sporadic AD (21 early onset and 86 late onset) from our memory clinic. Tests for (working)memory, language, executive function, mental speed and attention were administered. Global atrophy and global and lobar WMH were measured using 1 Tesla MRI. Linear regression analyses with terms for MRI measures, neuropsychological test results, age, sex, education, and the interaction between separate brain measures and age at onset were performed.

Results

Global atrophy was associated with more severely impaired global cognition, working memory, mental speed and executive function ($p < 0.05$). Significant interactions between global atrophy and age at onset showed that these associations were mostly attributable to patients with early onset AD. By contrast, an association between global atrophy and memory was found, which was specifically attributable to late onset AD patients. No associations between global WMH and cognitive function were found. Subsequently we analyzed regional WMH, and found that temporal WMH was associated with impaired memory, and frontal WMH was associated with slower mental speed.

Conclusion

Cortical atrophy, a key feature of AD, is linked to a wide range of cognitive functions, specifically in early onset AD patients. For WMH there were no interactions with age at onset, but we found specific associations between temporal WMH and memory and frontal WMH and mental speed.

2.3

| INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, characterized by gradually increasing cognitive impairment.[1] Underlying pathological processes in the brain, primarily atrophy, can be visualized and quantified using Magnetic Resonance Imaging (MRI). Cerebral atrophy in AD usually starts in the medial temporal lobe, extends to the remainder of the cortex and eventually results in global atrophy.[2-5] Global atrophy has been shown to be related to the severity of global cognitive impairment,[6-8] and to impairment of specific cognitive functions.[7-9]

White matter hyperintensities (WMH) are thought to reflect microvascular pathology.[10-12] In AD, WMH have been associated with impairment of executive functions[13-15] and memory.[15;16] These associations seem to be driven mainly by WMH located in the frontal and temporal lobes[13;14;16;17], but associations with parietal and occipital WMH have also been reported.[13;17] However, associations are modest, and other studies failed to find associations between WMH and cognition in AD.[6;18;19]

Discrepancies in the literature may partly be explained by differences in age at disease onset of the study sample. Both the degree of atrophy and the amount of WMH increase with age in healthy elderly. It is conceivable that this effect is different in AD patients, since patients with sporadic early onset AD are reported to have more global atrophy than patients with sporadic late onset AD, while WMH are typically observed in AD patients with late onset.[20;21]

In this study, we aimed to assess which cognitive functions are associated with global atrophy and with WMH in AD patients and whether these associations are modified by age at onset. We hypothesized that global atrophy would be associated with a wide variety of cognitive functions, whereas WMH would be more specifically associated with executive functions and memory, especially in the frontal and temporal lobes. Furthermore, we hypothesized that the associations between global atrophy and impairment of specific cognitive functions would be strongest in patients with early onset AD, since in those patients global atrophy is more widespread. By contrast, we expected the associations between WMH and executive and memory functions to be strongest in patients with late onset AD, since WMH is more widespread in those patients, probably contributing to their clinical picture.

| METHODS

Patients

We studied 107 patients with sporadic AD from the outpatient memory clinic of the Alzheimer Center of the VU University Medical Center (VUmc) with available 1T MRI of sufficient quality and available neuropsychological data, who had visited the memory clinic between May 2004 and September 2007. Patients with known mutations (N=1, Presenilin 1 mutation) and patients who withdrew their informed consent (N=1) were excluded from the study.

All patients underwent a standardized dementia assessment including medical history, informant-based history, physical and neurological examination, laboratory tests, neuropsychological testing, electroencephalogram (EEG), and magnetic resonance imaging (MRI) of the brain. Diagnoses of probable AD were made in a multidisciplinary consensus meeting according to the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) diagnostic criteria.[1] In addition, all patients met the NIA-AA criteria for probable AD dementia.[22] The level of education was classified using the 7-point rating scale of Verhage,[23] ranging from 1 (low, elementary school not completed) to 7 (high, University completed). Patients aged 65 years or less at diagnosis were considered to have early onset AD and patients above 65 years old were considered to have late onset AD. Vascular comorbidity (hypertension, diabetes mellitus, hypercholesterolaemia or myocardial infarction) was considered to be present when mentioned in the medical history, in case of hypertension when patients had a high blood pressure (systolic > 140 mm HG, diastolic > 80 mm Hg) measured during their visit, and/or when patients were using medication for the disease in question. The study was approved by the local Medical Ethical Committee. All patients gave written informed consent for their clinical data to be used for research purposes.

Neuropsychological tests

The neuropsychological test battery was designed to screen the major cognitive functions.[24] Briefly, the test battery included the following tests: the forward condition of *Digit Span* was used to assess attention (maximum score = 21).[25] Working memory was assessed with the backward condition of this test (max = 21). For memory, the *Visual Association Test* (VAT) was included (max = 12).[26] The naming condition of this test was used to assess language abilities (max. = 12). Furthermore, the *Trail Making Test* (TMT) was included in the test battery.[27] The simple part A provides a measure of mental speed and the more complex part B –and additionally part B minus A - a measure of executive functioning. Executive functions and language were additionally assessed using a test of Category Fluency (animals). The Mini Mental State Examination (MMSE) was included as a measure for global cognitive impairment.[28]

MRI

MRI was performed on a 1.0 Tesla machine (Magnetom Impact Expert Siemens AG, Erlangen, Germany) following a standard protocol, including axial FLAIR (fluid attenuated inversion recovery, 17 slices, FOV 250mm, matrix 256x256, slice thickness: 5mm, interslice gap: 1.5mm, TE: 105ms, TR: 9000ms, TI 2200ms, flip angle 180°), axial spin-echoT2-weighted images (21 slices, FOV 250mm, matrix 512x512, slice thickness 5mm, interslice gap 1.5mm, TE 119ms, TR 5775ms, flip angle 180°) and coronal T1-weighted 3D MPRAGE (magnetization prepared rapid acquisition gradient echo; 168 slices, field of view [FOV] 250mm, matrix 256x256, slice thickness 1.5mm, echo time [TE]: 7ms, repetition time [TR]: 15ms, inversion time [TI] 300ms, flip angle 15°).

Intracranial, whole brain and WMH volumes were automatically extracted following a previously described method.[29;30] Briefly, an intracranial mask was created based on an automatic template

based segmentation of the T2 images. The white matter (WM), grey matter, and cerebrospinal fluid (CSF) templates were registered to the T2 image. Next, the T2 and FLAIR images were co-registered, and the CSF segmentation was finalized (taking into account both the FLAIR and the T2 signal intensities).[31] Intracranial (IC) volumes and whole brain volumes (= IC - CSF volume) were extracted. As described elsewhere,[31] the automatic WMH segmentation is based on a Fuzzy interference system, which uses linguistic variables to classify a voxel. Using the Fuzzy C-Means algorithm,[32] each voxel of the FLAIR image was classified according to the voxel signal intensity (DARK, MEDIUM_BRIGHT and BRIGHT) and according to the voxel position (guided by the mapped templates; IC, WM). Accordingly, WMH were extracted based on location and signal intensity. The exact volumes of WMH were then computed. The anatomic regions of the frontal, temporal, parietal, and occipital lobes were determined as previously described.[33] Infratentorial hyperintensities were not taken into account.

To assess global atrophy, the following formula was used: (whole brain volume/IC volume)*1000. In a similar fashion, WMH volume was corrected for intracranial volume using the formula (WMH volume/IC volume)*1000.

CSF biomarkers

CSF biomarkers were assessed as markers of Alzheimer neuropathology. CSF was obtained by a lumbar puncture. Amyloid-Beta 1-42 ($A\beta_{1-42}$), total tau and tau phosphorylated at threonine-181 (p-tau) were measured by sandwich ELISA (Innotest β-amyloid[1-42], Innotest hTAU-Ag, and Innotest Phosphotau(181P); Innogenetics, Gent, Belgium).[34] CSF was available for 66 patients.

Statistical analysis

Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). To obtain a normal distribution, WMH volumes and TMT scores were logtransformed. Since WMH volumes can be zero and since zero-values can not be logtransformed, 0.01 was added to all corrected WMH volumes (corrected WMH-volume + 0.01) before logtransformation.

To assess associations between MRI measures (global atrophy and WMH: independent variables) and results on neuropsychological tests (dependent variables in separate models), we performed linear regression analyses. Age, sex and education were entered as covariates (model 1). In these models, the interaction between MRI measure and age group (early versus late onset) was then introduced. When the interaction was significant, the interaction term remained in the model and standardized beta's were estimated for early and late onset separately. When there was no interaction, the interaction term was removed from the model.

In model 2, the other brain measure was additionally adjusted for. In model 3, hypertension, diabetes mellitus, hypercholesterolaemia and myocardial infarction were entered as additional covariates. In model 4, CSF biomarker levels of $A\beta_{1-42}$, total tau, and p-tau were added to model 2 as additional covariates. In general, statistical significance was set at $p<0.05$. Interactions were considered significant if p-values were lower than 0.10.

In an additional analysis, we examined the relationships between regional WMH and neuropsychological tests using linear regression analyses with neuropsychological test score as dependent variable and age, sex, and education as covariates; frontal, temporal, parietal and occipital WMH volumes were entered stepwise forward as independent variables (model 1). Models 2 to 4 as defined above were repeated for regional WMH volumes.

| RESULTS

Demographic and clinical characteristics, MRI measures and neuropsychological test results are represented in table 1. Twenty-one patients had early onset AD (age=60±4), while 86 patients had late disease onset (age=75±5). Groups did not differ according to sex or education. The early onset group contained more smokers than the late onset group (p = .01). Patients with late onset had more global atrophy (p = .00) and larger WMH volumes (p = .00) than patients with early onset. WMH was most prominent in frontal and parietal lobes. Patients in both groups were mildly to moderately demented; in the early onset group, 29% had a CDR score of 0.5, while 62% scored 1 and 9% scored 2. In the late onset group, 32% of the patients scored 0.5 on the CDR, while 59% scored 1 and 9% scored 2. Patients with early onset had lower scores on MMSE (p = .02) and the backward condition of the digit span (p = .00) and higher scores on the category fluency test (p = .04) than patients with late onset AD.

Linear regression analyses showed that more global atrophy was related to lower scores on category fluency (table 2). Furthermore, significant interactions were found between global atrophy and age at onset in MMSE, the backward condition of the digit span, TMT parts A (Figure 1), B and B minus A. These interactions between atrophy and neuropsychological test implicated that more atrophy was related to worse scores in patients with early onset AD but not in patients with late onset AD. Larger WMH volumes were associated with better performance on VAT memory. Entering both MRI measures simultaneously (model 2) rendered essentially similar results, illustrating that global atrophy affected cognitive performance independently from WMH volume. The only exception was VAT memory, in which model 2 showed a significant interaction effect of global atrophy*onset. More global atrophy was related to worse memory performance in the late onset and not in the early onset patients. Repeating the analyses with additional adjustment for vascular risk factors (model 3) or CSF biomarker levels (model 4) did not change the results substantially.

Subsequently, we zoomed in on the regional distribution of WMH (table 3). Linear regression models with regional WMH (entering WMH in frontal, temporal, parietal and occipital lobes stepwise forward) showed that more temporal WMH was associated with worse performance on VAT memory and more frontal WMH was related to worse performance on TMT-A. Remarkably, more parietal WMH was associated with better performance on VAT memory.

Adjustments for global atrophy (model 2), vascular riskfactors (model 3) and CSF biomarkers (model 4) rendered similar results (Supplementary table 1; available online: http://www.j-alz.com/issues/35/vol35-1.html#supplementarydata03).

Table 1. Demographic and clinical characteristics.

	Total	Early onset AD	Late onset AD
Number of patients; N	107	21	86
Age	72 (8)	60 (4)	75 (5)*
Sex; n (%) women	61 (57%)	12 (57%)	49 (57%)
Education; median (range)[a]	5 (1-7)	5 (3-7)	5 (1-7)
Smoking, n (%)[b]	12 (12%)	6 (32%)	6 (8%)*
Systolic blood pressure, mm Hg[c]	144 (20)	139 (25)	145 (19)
Diastolic blood pressure, mm Hg[c]	85 (10)	86 (12)	85 (10)
Hypertension, n (%)	78 (73%)	14 (67%)	64 (74%)
Diabetes mellitus, n (%)	10 (9%)	1 (5%)	9 (11%)
Hypercholesterolaemia, n (%)	20 (19%)	4 (19%)	16 (19%)
Myocardial infarction, n (%)	4 (4%)	1 (5%)	3 (4%)
CSF amyloid-β_{1-42}, pg/ml[d, f]	483 (141)	440 (87)	501 (155)
CSF total tau, pg/ml[d, f]	738 (381)	728 (240)	742 (427)
CSF tau phosphorylated at threonine 181, pg/ml[d, f]	94 (37)	95 (24)	94 (41)
Intracranial volume, cc	1421 (122)	1412 (106)	1423 (127)
Whole brain volume, cc	1126 (104)	1146 (114)	1121 (101)*
WMH volume, cc	8 (10)	3 (3)	9 (11)*
Frontal WMH volume, cc	4 (5)	1 (2)	5 (6)*
Temporal WMH volume, cc	1 (1)	0 (0)	1 (1)
Parietal WMH volume, cc	2 (4)	1 (1)	3 (4)*
Occipital WMH volume, cc	1 (2)	1 (1)	1 (2)
CDR[e]	1 (0)	1 (0)	1 (0)
MMSE	22 (4)	20 (5)	22 (4)*
Digit span forward	11 (3)	11 (3)	11 (3)
Digit span backward	7 (3)	5 (3)	7 (2)*
VAT memory	5 (4)	4 (4)	5 (4)
VAT naming	11 (2)	11 (2)	11 (2)
TMT-A, seconds[g]	98 (81)	108 (86)	95 (80)
TMT-B, seconds[g]	363 (312)	384 (332)	358 (309)
TMT B minus A[g]	265 (236)	276 (249)	263 (234)
Category fluency	13 (5)	15 (5)	12 (5)*

Data are shown as mean (± sd) unless stated otherwise. Please note that raw values are shown, while statistical analyses were performed with values corrected for intracranial volume (WMH and global atrophy) and logtransformed values (WMH and TMT).
[a] Education classified according to the Dutch system of Verhage[23], ranging from 1 (low, elementary school not completed) to 7 (high, University). [b-e] Data available for 97[b], 102[c], 66[d], and 101[e] patients. [f] The following cut-off values are used in our laboratory: CSF Aβ_{1-42} <550 pg/mL, CSF tau>375 pg/mL, and p-tau >52 pg/mL. [34] [g] Lower scores signify better (i.e. faster) performance. WMH = White Matter Hyperintensities, MMSE = Mini Mental State Examination, VAT = Visual Association Test, TMT = Trailmaking Test
* difference between early onset and late onset group with p< .05

Table 2. Linear regression analyses.

		Global WMH	Global atrophy	Global atrophy in early onset	Global atrophy in late onset
MMSE	Model 1	-0.07	Interaction	-0.65*	-0.16
	Model 2	-0.09	Interaction	-0.69*	-0.15
	Model 3	-0.13	Interaction	-0.69*	-0.16
	Model 4	-0.05	Interaction	-0.69*	-0.08
Digit span forward	Model 1	-0.02	-0.10	-	-
	Model 2	-0.03	-0.10	-	-
	Model 3	-0.04	Interaction	-0.37	0.03
	Model 4	-0.04	-0.22	-	-
Digit span backward	Model 1	-0.08	Interaction	-0.48*	-0.06
	Model 2	-0.10	Interaction	-0.53*	-0.04
	Model 3	-0.16	Interaction	-0.55*	-0.05
	Model 4	-0.13	Interaction	-0.51*	-0.02
VAT memory	Model 1	0.22*	-0.21	-	-
	Model 2	0.21*	Interaction	0.07	-0.34*
	Model 3	0.15	Interaction	0.07	-0.36*
	Model 4	0.33*	Interaction	0.14	-0.38*
VAT naming	Model 1	-0.07	-0.14	-	-
	Model 2	-0.08	-0.14	-	-
	Model 3	-0.07	-0.14	-	-
	Model 4	-0.11	-0.05	-	-
TMT-A[1]	Model 1	0.20	Interaction	0.77*	0.17
	Model 2	0.22*	Interaction	0.86*	0.12
	Model 3	0.25*	Interaction	0.87*	0.14
	Model 4	0.22	Interaction	0.90*	-0.01
TMT-B[1]	Model 1	0.10	Interaction	0.73*	0.14
	Model 2	0.12	Interaction	0.79*	0.12
	Model 3	0.19	Interaction	0.78*	0.14
	Model 4	0.04	Interaction	0.83*	0.11
TMT-B minus A[1]	Model 1	0.03	Interaction	0.64*	0.13
	Model 2	0.04	Interaction	0.67*	0.12
	Model 3	0.13	Interaction	0.66*	0.14
	Model 4	-0.06	Interaction	0.67*	0.12

2.3

Table 2. Linear regression analyses. *(Continued)*

		Global WMH	Global atrophy	Global atrophy in early onset	Global atrophy in late onset
Category fluency	Model 1	-0.07	-0.32*	-	-
	Model 2	-0.09	-0.32*	-	-
	Model 3	-0.11	-0.34*	-	-
	Model 4	0.05	0.29	-	-

Model 1: Linear regression models with either global atrophy or WMH volume as independent variable, neuropsychological test score as dependent variable and age, sex and education as covariates. Model 2: model 1 with additional analysis for the other brain measure (global atrophy/WMH). Model 3: model 2 with vascular risk factors (hypertension, diabetes mellitus, hypercholesterolaemia and myocardial infarction) as additional covariates. Model 4: Model 2 with CSF biomarker levels of amyloid-ß 1–42 (Aß42), total tau, and tau phosphorylated at threonine-181 (P-tau-181) as additional covariates.

All data are presented as standardized β to allow comparison of effect sizes. When interactions between brain measure and age at onset were found, standardized β's per onset group are presented.
* p< .05
[1] Lower scores signify better (i.e. faster) performance. WMH = White Matter Hyperintensities, MMSE = Mini Mental State Examination, VAT = Visual Association Test, TMT = Trailmaking Test
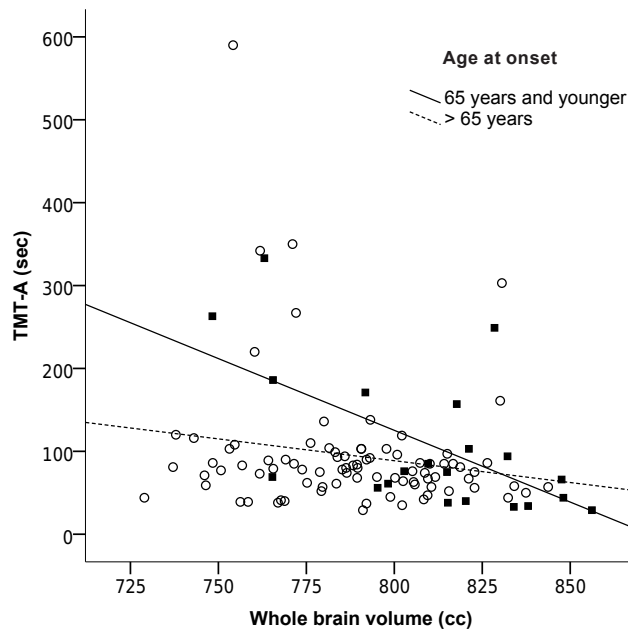
Figure 1. Scatter plot of whole brain volume (corrected for intracranial volume) and TMT-A scores. Filled squares indicate the early onset group and open circles indicate the late onset group. The lines indicate the relationship between whole brain volume and TMT-A. Linear regression analysis showed an interaction effect between whole brain volume and age at onset, with a significant association for the early onset group, but not for the late onset group.

Table 3. Linear regression analyses for regional WMH.

	Frontal WMH	Temporal WMH	Parietal WMH	Occipital WMH
MMSE	n.s.	n.s.	n.s.	n.s.
Digit span forward	n.s.	n.s.	n.s.	n.s.
Digit span backward	n.s.	n.s.	n.s.	n.s.
VAT memory	n.s.	-0.38*	0.55*	n.s.
VAT naming	n.s.	n.s.	n.s.	n.s.
TMT-A[1]	0.23*	n.s.	n.s.	n.s.
TMT-B[1]	n.s.	n.s.	n.s.	n.s.
TMT-B minus A[1]	n.s.	n.s.	n.s.	n.s.
Category fluency	n.s.	n.s.	n.s.	n.s.

Linear regression models with lobar WMH volumes as independent variables, neuropsychological test score as dependent variable and age, sex and education as covariates.
All data are presented as standardized β to allow comparison of effect sizes. * p< .05
[1] Lower scores signify better (i.e. faster) performance. WMH = White Matter Hyperintensities, MMSE = Mini Mental State Examination, VAT = Visual Association Test, TMT = Trailmaking Test

| DISCUSSION

We found that global atrophy was associated with more severe global cognitive impairment and more severely impaired working memory, mental speed and executive functions. These effects were mostly attributable to patients with early onset AD. Furthermore, we found an association between global atrophy and memory, which was attributable to the late onset group, and not the early onset group. There were no interactions between age at onset and WMH, but we found specific associations between temporal WMH and memory and frontal WMH and mental speed.

Previous studies have reported diverse findings with regard to the relationship between brain atrophy and cognitive function. In line with this study, some studies found a relationship between whole brain or cortical volume and global cognition in AD patients.[6-8;35] Studies of relationships between global atrophy and specific cognitive domains have reported associations with a wide range of cognitive domains, including attention,[8] working memory,[9] language,[7;9;36] memory,[8;9] visuoconstruction,[8;36] and executive functions.[7;8;36] Our findings of associations between global atrophy and global cognition, working memory, mental speed and executive functions fit within this range of reported associations. We took an innovative approach, as we focused on age at onset as possible effect modifier. In extension to previously reported findings that patients with early onset AD present with impairments in other cognitive functions than patients with late onset AD, who are more severely impaired in memory functions,[37-40] we found that global atrophy is associated with memory specifically in late onset AD patients. Most associations between global atrophy and cognitive functions, however, are attributable to patients with early onset AD and not to patients with late onset

AD. Although the sample size of the early onset group was relatively small, the effect sizes were far larger than in the late onset group. We speculate that global atrophy is linked in a more direct way to impairment of cognitive functions in early onset AD than in AD with late onset. This is in line with a previous study from our group, which reports that younger age, rate of global cortical atrophy and cognition are interrelated.[41]

Although the late onset AD patients showed cognitive impairment that is comparable in severity to early onset AD patients, these impairments could not be linked to global atrophy in such a direct way as in the younger patients. It seems that in older AD patients, variability in cognitive impairment is not only attributable to global atrophy and WMH. Perhaps, other factors besides AD related pathology play a role (e.g. comorbidity, depression and a less active lifestyle). Future research will have to show whether this is indeed the case. The finding that atrophy is more closely related to cognitive impairment in early onset than in late onset AD has clinical relevance as biomarkers including atrophy as seen on structural MRI, have been introduced as support for the diagnosis of probable AD dementia in the new NIA-AA criteria.[22] We have shown that the link between this biomarker and clinical symptoms differs between various groups of AD patients, which may be of importance for the implementation of the new diagnostic criteria.

With respect to the relationship between WMH and cognitive function, diverse associations have been reported, ranging from no relationship[6;14;18] to relationships with global cognition,[8;9;15;42;43] attention,[8;36] mental speed,[7;15] working memory,[15] language,[9;15;36] calculation,[36] memory,[8;9;15;16] tactile gnosis,[44] visuoconstruction,[8] and executive functions.[7-9;13;15] In the present study, global WMH was hardly related with cognitive function, but we did find specific regional associations; temporal WMH was related to memory, and frontal WMH to mental speed. Contrary to our expectations, we found that more WMH, specifically located in the parietal lobes, was associated with less impaired memory performance. We are not certain how to explain this, but one should bear in mind that all patients had AD. Similar surprisingly negative associations between WMH and cognitive impairment have been reported before by others.[45] Our finding may possibly be explained by the hypothesis that patients with prominent parietal WMH have a more non-memory profile. These patients may resemble a subtype of AD patients, including patients with posterior cortical atrophy (PCA), who show specific impairment of the visuospatial, visuoperceptual, praxis, calculation, and spelling abilities. Their memory abilities seem to be relatively spared, while other cognitive functions are more severely impaired. We have described associations between a non-memory profile and parietal damage in AD patients in a recent study, which reports that increased amyloid burden together with metabolic dysfunction, in the parietal lobe of younger patients with AD, may contribute to the distinct cognitive profile in these patients.[46] However, it should be noted that all brain lobes, including the parietal lobe, are in some way involved with memory processes.[47]

There was no interaction between age at onset and WMH, indicating that the found associations are similar for early onset and late onset patients. As has been described

previously, the distribution of WMH is strongly skewed to the right, with many individuals having very little or no WMH, and a minority having severe WMH.[10;48] It seems that even though the number of early onset patients with WMH is lower than the number of late onset patients with WMH, if WMH are present in these early onset patients, they have a similar effect on cognition as in older patients.

Although much research has been done regarding the relationship between brain measures and cognition in dementia, as far as we know this study is the first to investigate the modifying influence of age at onset on these associations. Other strengths include the use of a homogeneous sample of only patients with probable AD dementia. Many of the previous studies included a more heterogeneous sample ranging from healthy controls to patients with MCI and patients with different types of dementia, whereas in this study, only AD patients were included. Since it is plausible that variations in brain changes and in other factors influencing cognition differ between these patient groups, limiting oneself to a more homogeneous sample may give a clearer insight in the studied relationships which may be specific for AD, and not other dementias. Furthermore, we used a volumetric method to quantify atrophy and WMH, whereas some other studies used visual rating scales. Since visual rating scales are more crude, subtle differences in brain changes between patients may remain unnoticed and relationships with cognition may not be reflected accurately.

Amongst the limitations of this study is the relatively low field strength of 1 Tesla of the MRI used to acquire the images. Future studies at higher field strengths -but also using new MRI techniques such as Diffusion Tensor Imaging to study white matter tracts and PET techniques to study brain metabolism- will allow a deeper understanding of the relationships between brain changes and their specific effects on cognitive performance in AD patients with early and with late onset.

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2.3

Associations between MRI measures and neuropsychological impairment in early and late onset Alzheimer's disease

Aus der Heimat hinter den Blitzen rot

From my homeland, behind the red flashes of lightning

Da kommen die Wolken her,

That's where the clouds come from,

Aber Vater und Mutter sind lange tot,

But Father and Mother are long dead;

Es kennt mich dort keiner mehr.

No one there knows me anymore.

Wie bald, ach wie bald kommt die stille Zeit,

How soon, ah, how soon will that quiet time come,

Da ruhe ich auch, und über mir

When I too shall rest, and over me

Rauscht die schöne Waldeinsamkeit,

the beautiful forest's loneliness shall rustle,

Und keiner kennt mich mehr hier.

And no one here shall know me anymore.

In der Fremde
Robert Schumann

3

RATE OF COGNITIVE DECLINE
IN ALZHEIMER'S DISEASE

3.1

MOST RAPID COGNITIVE DECLINE IN APOE ε4 NEGATIVE ALZHEIMER'S DISEASE WITH EARLY ONSET

A.E. van der Vlies[1], E.L.G.E. Koedam[1], Y.A.L. Pijnenburg[1], J.W.R. Twisk[2], P. Scheltens[1], W.M. van der Flier[1]

[1] Alzheimer Center & Department of Neurology, Neuroscience Campus Amsterdam,
[2] Department of Clinical Epidemiology and Statistics, VU University Medical Center, Amsterdam, the Netherlands.

| ABSTRACT

Background

We aimed to compare the rate of cognitive decline in patients with early and late onset Alzheimer's Disease (AD) and to investigate the potentially modifying influence of APOE genotype.

Methods

We included 99 patients with early onset AD (age ≤65 years) and 192 patients with late onset AD (age >65 years) who had at least 2 MMSE scores (range 2-14) obtained at least one year apart. Linear mixed models were performed to investigate the rate of cognitive decline dependent on age at onset and APOE genotype.

Results

Mean (sd) age for patients with early onset was 57.7 (4.5) years, and 74.5 (5.1) for patients with late onset. Age at onset was not associated with baseline MMSE (β(SE)= 0.8 (0.5), p= 0.14). However, patients with early onset showed faster decline on the MMSE of (β(SE)) 2.4 (0.1) points/year, whereas those with late onset showed decline of 1.7 (0.1) points/year (p= .00). After stratification according to APOE genotype, APOE ε4 non-carriers with early onset showed faster cognitive decline than non-carriers with late onset (2.4 (0.3) vs 1.3 (0.3) points/year, p= .01). In APOE ε4 carriers, no difference in rate of cognitive decline was found between patients with early and late onset (β(SE)= 0.2 (0.2), p= 0.47).

Conclusion

Patients with early onset AD show more rapid cognitive decline than patients with late onset, suggesting that early onset AD follows a more aggressive course. Furthermore, this effect seems to be most prominent in patients with early onset that do not carry the genetic APOE ε4 risk factor for AD.

| INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, characterized by gradually increasing cognitive impairment.[1] Typically, AD is regarded as a disease occurring at old age and estimates of prevalence and incidence do indeed increase exponentially with advancing age.[2;3] However, AD also affects younger people (under the age of 65 years), commonly referred to as early onset AD. Although it has been suggested that early onset AD follows a more aggressive course than late onset AD,[4;5] only few studies have actually addressed this possible difference and results are conflicting, with one study showing a faster cognitive decline in young,[6] and the other in older patients.[7]

The apolipoprotein E gene (APOE) is an important risk factor for AD.[8-11] Presence of the ε4 allele increases the risk of AD and has been associated with an earlier age at onset, although this association may be less pronounced in patients under the age of 65 years.[8;10;12-14] Conflicting results have been found with regard to the relation between APOE ε4 genotype and rate of cognitive decline in AD patients, with studies reporting slower,[15;16] faster,[17] and the same[18;19] rate of cognitive decline in APOE ε4 carriers compared to non-carriers.

The aim of this study was to compare the rate of general cognitive decline as measured by the Mini-Mental State Examination (MMSE)[20] between AD patients with early disease onset and those with late disease onset. Furthermore, we investigated the additional influence of APOE genotype on the rate of cognitive decline.

| METHODS

Subjects

We studied consecutive patients with sporadic AD from the outpatient memory clinic of the Alzheimer Center of the VU University Medical Center (VUmc). At baseline, all patients had undergone a standardized dementia assessment including medical history, informant-based history, physical and neurological examination, laboratory tests, neuropsychological testing, electroencephalogram (EEG) and magnetic resonance imaging (MRI) of the brain. Diagnoses of probable AD were made in a multidisciplinary consensus meeting according to the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) diagnostic criteria.[1] Patients aged 65 years or less at diagnosis were considered to have early onset AD and patients above 65 years old were considered to have late onset AD. The duration of the cognitive complaints as reported by the patient and/or caregiver were recorded to estimate the disease duration at the time of diagnosis. To be included in this study patients had to have at least 2 MMSE scores obtained no less than one year apart. The resulting data set included 1194 MMSE scores from 291 patients. Follow-up time varied between one and six years (mean = 2.3; sd= 1.1) and patients had a median of 3 visits (range: 2-14). The study was approved by the local Medical Ethical Committee. All patients gave written informed consent for their clinical data to be used for research purposes.

APOE

APOE genotype was determined according to the following procedure. DNA was isolated from 10 ml EDTA blood. APOE genotype was determined at the Department of Clinical Chemistry of the VUmc with the Light Cycler APOE mutation detection method (Roche Diagnostics GmbH, Mannheim, Germany). APOE data were available for 185 patients (early onset: n= 69; late onset: n= 116) and were analyzed according to the presence or absence of an APOE ε4 allele.

Statistical analysis

Statistical analyses were performed using SPSS 15.0. For group comparisons at baseline, χ^2-tests, Mann-Whitney tests and t-tests were used when appropriate. Linear mixed models were used to assess associations between age at onset ($\leq$ 65 years or > 65 years) and the rate of cognitive decline as measured by MMSE. This approach has increased statistical power as it accounts for within-person correlations over time, allows different numbers of assessments, and accounts for varying time intervals between assessments. All assessments, including baseline, were taken into account. A random intercept was assumed, i.e. baseline MMSE was allowed to vary between patients. The first model included terms for age at onset, time, sex, and the interaction between age at onset and time, with MMSE score as dependent variable. From this model, baseline MMSE and annual change in MMSE for young and old patients could be estimated. The analysis was repeated after stratification according to APOE ε4 status, so that the effect of age at onset on decline in MMSE over time could be estimated separately for APOE ε4 carriers and non-carriers.

| RESULTS

Ninety-nine (34%) patients had early onset AD and 192 (66%) had late onset AD (table 1). Mean age for the patients with early onset was 57.7 years, for the patients with late onset it was 74.5 years. Groups did not differ according to sex. Patients with early onset AD had longer disease duration than patients with late onset AD (t = 2.1, p = .04). No differences in baseline MMSE were found according to age at onset (t = -1.7, p = .10).

We used linear mixed models to compare annual change in MMSE dependent on age at onset ($\leq$ 65 years or > 65 years). Age at onset was not associated with baseline MMSE (β(SE)= 0.8 (0.5), p = .14). However, an interaction between age at onset and time (β(SE)= 0.6 (0.2), p = .00) was found. AD patients with early onset were prone to a faster disease progression (estimated decline in MMSE = -2.4 (SE= 0.1) points per year) than late onset AD patients (-1.7 (SE= 0.1) points per year).

Subsequently, patients were stratified according to APOE genotype, to assess the effect of age at onset on MMSE score over time for APOE ε4 carriers and non-carriers separately (Figure 1). Of the non-carriers, 22 (43%) had early disease onset and 29 (57%) had late onset.

Table 1. Baseline demographic and clinical characteristics.

	Early onset AD	Late onset AD
N	99	192
Sex, n (%) female	50 (51%)	96 (50%)
Age at diagnosis, mean (sd)	57.7 (4.5)	74.5 (5.1)**
Duration (years), mean (sd)	3.2 (2.0)	2.7 (2.2)*
APOE ε4 positives, n (%)[a]	47 (68%)	87 (75%)
MMSE at baseline, mean (sd)	21.1 (4.5)	22.0 (4.0)
Follow-up time (years), mean (sd)	2.5 (1.2)	2.3 (1.0)*
Number of visits, median (range)	4 (2-10)	3 (2-14)

MMSE = Mini Mental State Examination
[a] APOE data available for 185 patients
* p< .05, ** p<.01
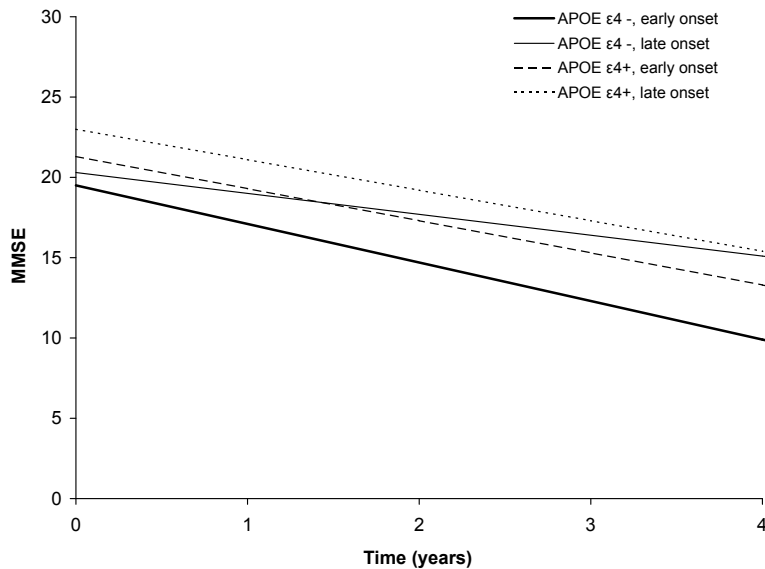
Figure 1. Estimated regression lines of MMSE-score by time for early onset AD and late onset AD, stratified according to APOE genotype. In APOE ε4 carriers, early and late onset patients show similar rates of cognitive decline. By contrast, in non-carriers, early onset patients show faster rate of cognitive decline than late onset patients.

Linear mixed models showed no association of age at onset with baseline MMSE (β(SE)= 0.9 (1.2), p = 0.45), but an interaction between age at onset and time (β(SE)= 1.1 (0.4), p = .01) was found. AD patients with early onset were prone to a faster disease progression (estimated decline in MMSE = 2.4 (SE= 0.3) points per year) than late onset AD patients (1.3 (SE= 0.3) points per year)

Of the APOE ε4 carriers, 47 (35%) patients had early disease onset and 87 (65%) had late onset. Age at onset was associated with baseline MMSE (β(SE)= 1.7 (0.8), p = 0.05). Patients with early onset had lower baseline MMSE (β(SE)= 21.3 (1.3)) than patients with late onset (β(SE)= 23.0 (1.3)). There was no interaction between age at onset and time, implying similar decline over time (β(SE)= 0.2 (0.2), p = 0.47).

| DISCUSSION

We found that AD patients with early disease onset showed faster cognitive decline than patients with late onset, while patients with early and late onset AD did not differ according to disease severity at the time of diagnosis. This provides support for the hypothesis that early onset AD runs a more aggressive course than late onset AD. Moreover, we found that the effect of age at onset was mostly attributable to APOE ε4 non-carriers. APOE ε4 non-carriers with early onset showed faster cognitive decline than non-carriers with late onset AD. This effect was not found in APOE ε4 carriers; patients with early and late onset showed similar rates of cognitive decline.

Based on clinical observations, it has been suggested that young AD patients show faster cognitive decline than older AD patients. However, actual studies on the difference in cognitive decline between AD patients with early onset and those with late onset are scarce and their conclusions are conflicting. Our findings are consistent with a previous study in a relatively small sample, in which young AD patients showed faster decline on an extended version of the MMSE (max. score = 57) than older AD patients.[6] By contrast, another study reports a faster rate of cognitive decline in late onset AD patients.[7] However, the number of patients with follow-up data in this study was relatively small and the distribution of patients according to age at onset was unclear. Furthermore, the authors state that although rate of progression was more rapid in late onset AD, considerable overlap among values for the two patient groups was observed, which led them to conclude that age at onset is not a strong predictor of rate of progression of dementia in patients with AD. The present study contains a large sample of AD patients with considerable follow-up, and a clear effect of age at onset on the rate of cognitive decline was shown.

Furthermore, we have shown that the effect of age at onset on the rate of cognitive decline is attributable to APOE ε4 non-carriers, and not so much to APOE ε4 carriers. In non-carriers, early onset patients show a doubled rate of cognitive decline compared to late onset patients, while younger and older APOE ε4 carriers have a similar rate of decline. The conflicting results with regard to the relation between APOE genotype and rate of cognitive decline in AD patients which have been reported by previous studies, may well be explained by this effect. Since these previous studies either included only late onset AD patients,[15;19] or made no distinction between patients with early and late onset,[16-18] they may have missed the rather sensitive modifying effect of APOE genotype.

Our finding of early onset APOE ε4 non-carriers showing the fastest cognitive decline is in line with a previous study by our group that reported that younger age, absence of APOE ε4, and low MMSE at baseline were associated with higher whole-brain atrophy rate.[21] It is feasible that patient with early onset and absence of APOE ε4 not only show a faster cognitive decline but also have a higher rate of whole-brain atrophy. Although the presence of APOE ε4 is a risk factor for developing AD, our data suggest that, especially in patients with early onset, this same presence protects patients, once they actually have the disease.

It is possible that the patients with early onset AD presented at our memory clinic when they had progressed further into the disease process than the late onset AD patients. This would explain their faster cognitive decline, since baseline disease severity has been shown to predict the rate of subsequent cognitive decline.[22;23] However, we do not think this explains our findings, as the disease severity as measured by the MMSE at baseline was similar for patients with early onset AD and late onset AD.

The question whether early and late AD should be considered as two subtypes of the same disorder or as two separate disorders is still under debate.[24] It should be noted that the age of 65 years used in this and most other studies to distinguish early disease onset from late onset is arbitrary. It has been shown that early and late onset AD patients share the presence of amyloid plaques, neurofibrillary tangles and neuronal loss, which indicates the same underlying neuropathology.[4;25] However, the difference we found in rate of cognitive decline between both groups, especially in APOE ε4 non-carriers, might be considered as an argument to regard early and late onset AD as two entities, with specific characteristics. It might be speculated that in some AD patients, the disease process is so aggressive that it becomes apparent early in life, resulting in early onset of the disease. The faster rate of cognitive decline in young patients might then be explained by this more aggressive process.

Among the limitations to this study is the fact that no post mortem data were available. Therefore, it cannot be excluded that some patients were misdiagnosed as having AD, which might have contaminated the data. At baseline, however, all patients were clinically characterized in a uniform manner, and all patients fulfilled clinical criteria for AD. Another limitation is the fact that age at onset is clearly confounded by the patients biological age. In this data set, it is not possible to tackle the associations between the two. However, considering biological age in relation to change in cognition, one would expect older patients to show more cognitive impairment, since it is well known that even healthy persons of old age show more cognitive decline than younger healthy persons. In this study, the opposite effect was found in AD patients: the patients with early onset showed more cognitive decline than the patients with late onset. Strengths of the current study include the relatively large group of AD patients that was followed for an average duration of over two years. Furthermore, the applied statistical method of linear mixed models allowed use of all available MMSE's, up to 14 per patient.

In conclusion, we found that patients with early onset AD deteriorate faster than AD patients with late onset. The effect of age at onset differs between APOE ε4 carriers and non-carriers. In APOE ε4 carriers, patients with early and late onset show similar rates of decline. However, APOE ε4 non-carriers with early onset show a far steeper cognitive decline than non-carriers with late onset. This adds to the notion that AD is to be considered as a heterogeneous disorder with regard to clinical features and disease course. An aggressive disease course is most prominent in patients with early onset AD that do not carry the genetic risk factor for AD.

3.1

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3.1

3.2

CSF BIOMARKERS PREDICT RATE OF COGNITIVE DECLINE IN ALZHEIMER'S DISEASE

M.I. Kester[1], A.E. van der Vlies[1], M.A. Blankenstein[2], Y.A.L. Pijnenburg[1], E.J. van Elk[2], P. Scheltens[1], W.M. van der Flier[1]

[1] Alzheimer Center & Department of Neurology, Neuroscience Campus Amsterdam, [2] Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands.

| ABSTRACT

Objective

CSF biomarkers amyloid beta 1-42 ($A\beta_{1-42}$), total tau (tau) and tau phosphorylated at threonine 181 (p-tau) are useful diagnostic markers for Alzheimer's disease (AD). Less is known about these biomarkers as predictors for further cognitive decline in patients with AD. We hypothesized that high tau, especially in combination with relatively low p-tau, is a marker of rapid decline, since it has been associated with fast neuronal degeneration.

Methods

151 AD patients, of whom we had baseline CSF, were included from our memory clinic. All patients had at least two Mini Mental State Examination (MMSE) scores, obtained no less than one year apart. Linear mixed models were used to assess associations between CSF biomarkers and the rate of cognitive decline as measured with the MMSE. CSF biomarkers were used in quintiles, random intercept and random slope with time were assumed and the analyses were corrected for sex and age.

Results

The AD patients (45%F, 66±9years, baseline MMSE 22±4) had a follow-up period of 2.0(1.0-5.0) years. Linear mixed models revealed no relations between any CSF biomarker and baseline MMSE. However, CSF biomarkers did predict cognitive decline over time. A low p-tau/tau-ratio was the strongest predictor with a dose dependent effect (lowest vs highest quintile: 2.9 vs 1.3 MMSE-points annual decline, p for trend <0.001). In addition, low $A\beta_{1-42}$, high tau and high tau/ $A\beta_{1-42}$-ratio were also associated with rapid cognitive decline (p<0.05).

Conclusion

At the time of diagnosis a combination of high CSF tau without proportionally elevated p-tau is associated with a faster rate of cognitive decline.

3.2

| INTRODUCTION

CSF biomarkers amyloid beta 1-42 (A$\beta_{1\text{-}42}$), total tau (tau) and tau phosphorylated at threonine 181 (p-tau) reflect the neuropathology of Alzheimer's disease (AD) and are useful as diagnostic markers for Alzheimer's disease (AD). Furthermore, they are associated with progression of mild cognitive impairment (MCI) to AD.[1-3] Less is known about these biomarkers as predictors for further cognitive decline in patients with AD. Results from several cross-sectional studies examining the relation between CSF A$\beta_{1\text{-}42}$ or tau and Mini Mental State Examination (MMSE) are conflicting.[4-10] Only a few longitudinal studies relating CSF biomarkers to MMSE changes were performed, with negative results. However, these studies had limited statistical power.[11;12]

CSF tau has been suggested as a general marker for neuronal degeneration.[13] Phosphorylated tau (p-tau) is a more specific marker for AD, as it has been associated with neurofibrillary tangles, that mainly exist of tau in the phosphorylated state.[14] The combination of high tau with normal p-tau has been associated with aggressive diseases, like Creutzfeldt-Jakob disease and stroke.[15-17] In these disorders there is a high rate of neuronal cell death, but hardly any phosphorylation.

In light of the above, we hypothesized that high CSF tau levels, and especially in combination with less elevated CSF p-tau, as represented by a low p-tau/tau ratio, would predict rapid cognitive decline. For this cause, we aimed to investigate the associations of baseline CSF biomarker levels of A$\beta_{1\text{-}42}$, tau and p-tau and their ratios tau/ A$\beta_{1\text{-}42}$, p-tau/ A$\beta_{1\text{-}42}$ and p-tau/ tau, with deterioration on MMSE in a large cohort of AD patients.

| METHODS

Patients

We included 151 patients with AD, of whom we had baseline CSF results, from our memory clinic. All patients underwent a standard dementia screening including physical and neurological examination as well as laboratory tests, EEG and brain MRI. Baseline cognitive screening included a MMSE and usually involved comprehensive neuropsychological testing. The diagnosis of probable AD was made according to the NINCDS-ADRDA criteria[18] by consensus of a multidisciplinary team, without knowledge of CSF results. Of all patients we had information about education, classified using the Verhage scale.[19] APOE genotype was determined in 130 patients as described previously.[20] The study was approved by the local ethical review board and all subjects gave written informed consent (consent for research).

Follow-up

All patients had at least one follow-up, no less than one year after baseline. At follow-up the MMSE was used as a measure of general cognitive function. Median number of MMSE tests within one patient was four, with a minimum of two and a maximum of fourteen.

The total number of MMSE tests that were included in the analyses was 648. Duration of follow-up was 2.0 (range 1.0-5.0) years. The median number of MMSE tests per year was two, which means that most patients had a MMSE test every half year.

CSF analysis

CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 10 mL polypropylene tubes. Within two hours, CSF samples were centrifuged at 1800 g for 10 minutes at 4°C. A small amount of CSF was used for routine analysis, including total cells (leucocytes and erythrocytes), total protein and glucose. CSF was aliquoted in polypropylene tubes of 0.5 or 1 ml and stored at -80°C until further analysis. CSF $A\beta_{1-42}$, tau and p-tau were measured with Innotest sandwich ELISA as described previously.[21] The team involved in the CSF analysis was not aware of the clinical diagnosis. As the manufacturer does not supply controls, the performance of the assays was monitored with pools of surplus CSF specimens. In the study period multiple specimens with various concentrations, which were included in seven to eighteen runs, have been used for this purpose. The inter-assay coefficient of variation (mean±SD) was 11.3±4.9% for $A\beta_{1-42}$, 9.3±1.5% for tau and 9.4±2.5% for p-tau.

Statistical analysis

For statistical analysis, SPSS version 15.0 (for Windows) was used. CSF variables ($A\beta_{1-42}$, tau and p-tau, and their ratios tau/ $A\beta_{1-42}$, p-tau/ $A\beta_{1-42}$ and p-tau/tau) were recoded in quintiles for the analyses, as they were not normally distributed. Linear mixed models were applied to assess the associations between baseline CSF biomarkers and cognitive decline as measured with the MMSE. A linear mixed model has increased statistical power as it accounts for within-person correlations over time, allows different numbers of assessments, and accounts for varying time intervals between assessments. All assessments, including baseline, were taken into account. A random intercept and a random slope with time (in years) were assumed, meaning that the model accounted for individual variation of baseline MMSE and variation in change in MMSE over time. The model included terms for one of the CSF biomarkers ($A\beta_{1-42}$, tau, p-tau or their ratios), time, the interaction between the CSF biomarker and time, sex, age and education. All data are represented as β(SE). First analyses were performed with CSF quintiles as continuous variables, to estimate general associations. In the model the term for the CSF biomarker represents the cross-sectional effect: a difference in baseline MMSE for every quintile of the used biomarker. The interaction term of CSF biomarker * time represents the longitudinal effect: an increase of annual MMSE change for every biomarker quintile. Secondly, we used the CSF biomarker quintiles as categories, to estimate annual decline in MMSE for each quintile; these models were also corrected for sex, age and education. Additionally, for the patients with available APOE genotype data, we have repeated the analyses with the CSF quintiles as continuous variables with an extra term for APOE genotype (APOE ε4 allele present or absent).

In an additional analysis, we assessed the effect of having a "normal" CSF profile. An AD CSF profile was defined as 2 or 3 abnormal biomarkers, by contrast 0 or 1 abnormal biomarkers

were defined as a normal CSF profile. Levels of CSF $A\beta_{1-42}$ <495 pg/ml, CSF tau >356 pg/ml and p-tau >54 pg/ml were considered abnormal.[22]

| RESULTS

Forty-five percent of the AD patients were female, they were aged 66±9 years and had a baseline MMSE of 22±4, as shown in table 1. The average rate of cognitive decline in our population was 2.2 (SE 0.3) MMSE points per year.

Table 1. Baseline characteristics.

	AD patients (n=151)
Gender = woman, n (%)	68 (45%)
Age, median (range)	67 (41-94)
Baseline MMSE	22±4
Level of education,* median (range)	5 (1-7)
Follow-up period, years (range)	2.0 (1.0-5.0)
APOE ε4 allele present,# n (%)	93 (72%)
$A\beta_{1-42}$, pg/ml	414 (337-493)
Tau, pg/ml	658 (442-898)
P-tau, pg/ml	86 (66-112)
Previous medical history:	
Hypertension, n (%)	22 (15%)
Hypercholesterolemia, n (%)	16 (11%)
Myocardial infarction, n (%)	8 (5%)
Diabetes Mellitus, n (%)	4 (3%)
Stroke, n (%)	2 (1%)
Parkinson's disease, n (%)	2 (1%)

Data are represented as mean±SD or median (interquartile range) unless indicated otherwise.
AD= Alzheimer's disease, $A\beta_{1-42}$= amyloid beta 1-42, tau= total tau, p-tau= tau phosphorylated at threonine 181,
* Education using Verhage's classification.[19], # APOE ε4 genotype known in 130 patients

We used linear mixed models to investigate associations between CSF biomarkers $A\beta_{1-42}$, tau, p-tau and their ratios with baseline MMSE, and annual change in MMSE. Age, sex and education adjusted analyses were performed with the CSF biomarkers in quintiles as continuous variables (table 2). None of the CSF biomarkers was associated with baseline MMSE. By contrast, low baseline $A\beta_{1-42}$ and high tau levels predicted higher annual decline in MMSE ($p < 0.05$). A low p-tau/tau ratio was the strongest predictor of rapid cognitive decline ($\beta(SE)=0.42(0.12)$, $p < 0.001$). In addition, a high ratio of tau/Aβ42 ratio was also associated with cognitive decline ($p < 0.05$). Neither p-tau nor the ratio of p-tau/ $A\beta_{1-42}$ were associated with annual change in MMSE. For 130 patients the APOE genotype was known.

After adjustment for APOE genotype (in addition to sex, age and education) the results remained the same ($A\beta_{1-42}$ β(SE)= 0.30(0.13), p<0.05; tau β(SE)= -0.28(0.13), p<0.05; tau/$A\beta_{1-42}$ ratio β(SE)= -0.29(0.13), p<0.05; p-tau/tau ratio β(SE)= 0.48(0.13), p<0.001).

Table 2. CSF biomarkers as predictors for cognitive deterioration on MMSE.

CSF biomarkers	Estimated effects on baseline MMSE	Estimated effects on annual MMSE change
$A\beta_{1-42}$	0.28 (0.22)	0.27 (0.12)*
Tau	-0.09 (0.21)	-0.25 (0.12)*
P-tau	-0.04 (0.22)	-0.15 (0.12)
Tau / $A\beta_{1-42}$ ratio	-0.14 (0.22)	-0.27 (0.12)*
P-tau / $A\beta_{1-42}$ ratio	-0.11 (0.22)	-0.18 (0.12)
P-tau / tau ratio	0.34 (0.22)	0.42 (0.12)**

Data are represented as β(SE). Linear mixed models were used to assess the association between CSF biomarkers (or their ratio) and the rate of cognitive decline as measured with the MMSE. A random intercept and random slope for time (in years) were assumed. The model included terms for CSF biomarker (or their ratio), time, the interaction between CSF biomarker and time, sex, age and education. Analyses were done for quintiles of CSF biomarkers (or their ratio) to obtain normal distribution. The given β represents in the first column the difference in baseline MMSE per biomarkers quintile and in the second column the difference in annual MMSE change per biomarker quintile.
AD= Alzheimer's disease, MMSE= mini mental state examination, $A\beta_{1-42}$= amyloid beta 1-42, tau= total tau, p-tau= tau phosphorylated at threonine 181
* p<0.05
** p<0.001

To assess whether the observed effects were dose response relations or were attributable to a threshold effect, we entered the biomarkers quintiles as categorical variables (adjusted for sex, age and education). Annual decline in MMSE by biomarker quintiles is shown in figure 1, for associations that were significant when biomarkers were entered as continuous variables. The strongest predictive value for cognitive decline was found for the p-tau/tau ratio. For this ratio the lowest quintile was associated with an annual rate of decline of 2.9 points, while a ratio in the highest quintile was associated with an annual decline of 1.3 points in MMSE. Thus, patients with very high tau and less elevated levels of p-tau (lowest quintile of p-tau/tau ratio) showed very rapid cognitive decline. By contrast, patients with lower levels of tau in combination with higher levels of p-tau remained relatively stable. The figure illustrates that this effect was dose dependent.

There were 15 patients with a normal CSF profile and 136 patients with an AD CSF profile. In an additional analysis, we compared cognitive decline according to CSF profile. Patients with a normal CSF pattern declined 1.5 (SE 0.5) MMSE points per year, while patients with an AD CSF profile had a decline of 2.2 (SE 0.6) MMSE points annually (p=0.15).
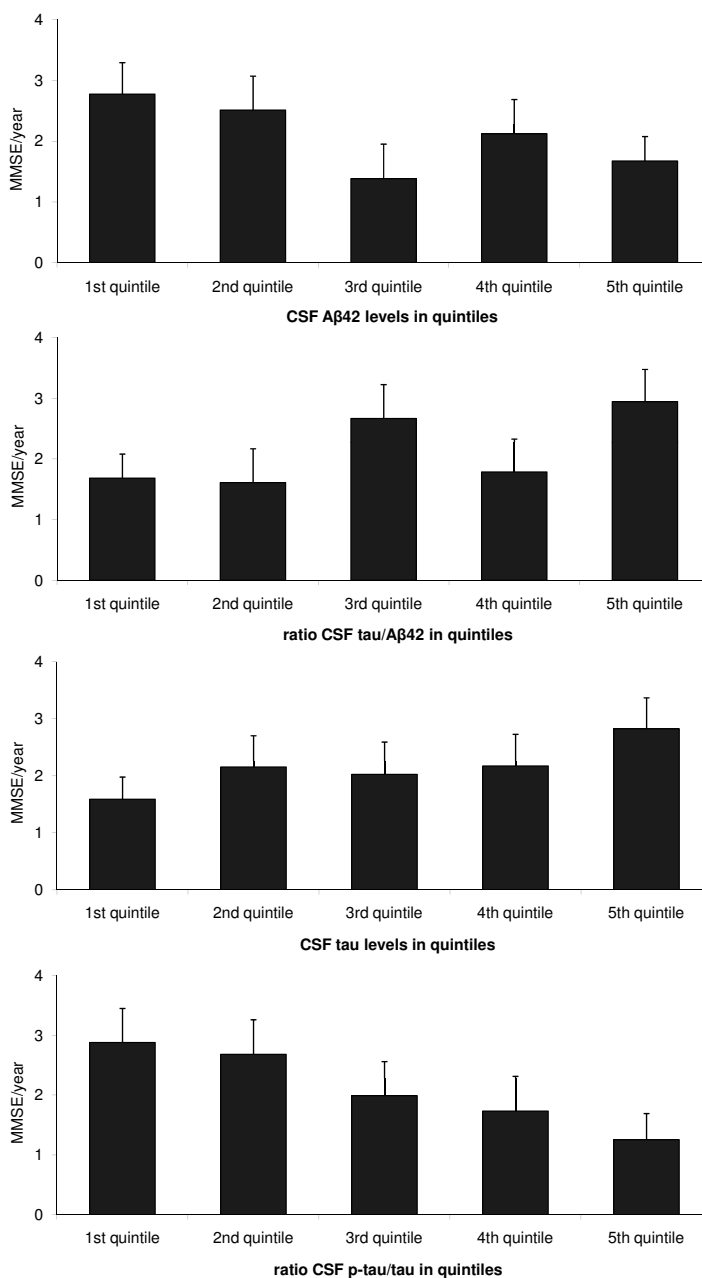
Figure 1. Linear mixed models were used to investigate the association between CSF biomarkers and the rate of cognitive decline as measured with the MMSE. A random intercept and random slope for time (in years) were used. In addition the model included terms for sex, age, education, CSF biomarker (or their ratio) and the interaction between CSF biomarker and time. CSF biomarker quintiles were entered as categories to obtain estimated annual change for every quintile. Cognitive deterioration was defined in this figure as positive annual change in MMSE, thus higher annual change indicates faster progression. AD= Alzheimer's disease, MMSE= mini mental state examination, $A\beta_{1-42}$= amyloid beta 1-42, tau= total tau, p-tau= tau phosphorylated at threonine 181.

| DISCUSSION

We found high tau in combination with less elevated p-tau, as represented by a low p-tau/tau ratio, to be the strongest predictor for cognitive decline over time in AD. A low p-tau/tau ratio was associated with faster annual cognitive decline than a high ratio (2.9 vs 1.3 MMSE points per year). Low $A\beta_{1-42}$ levels, high tau levels and a high tau/ $A\beta_{1-42}$ ratio were also associated with more rapid cognitive deterioration.

Most studies found no relation between CSF biomarkers and cognition within AD samples, probably because they had a cross-sectional design.[4-9;11;12] The few studies with a longitudinal design, either had a very small group of patients or had a short period of follow-up.[11;12] In the present longitudinal study, we found a strong predictive effect for CSF biomarkers on cognitive decline. In our center lumbar puncture is performed on a routine basis in the majority of patients presenting with memory problems. In addition, most AD patients receive treatment and cognitive follow-up at our center. The combination of these two factors resulted in a large representative group of AD patients with a relatively long period of follow-up. By using linear mixed models, we were able to include all available MMSE measurements. Previously, we found that patients with high tau and p-tau levels had severe impairments of especially memory, mental speed and executive functions.[23] For this reason, we feel that it would be very interesting to examine whether the revealed relations between cognition and CSF biomarkers, are mainly associated with certain domains. However, as a measurement of cognition in the follow-up period, only MMSE data and no extensive neuropsychological data were available for a large number of patients. In the future we hope to gather enough data to be able to analyze specific cognitive domains. Another limitation of our study could be the absence of post-mortem verification of the AD diagnosis. It may be questioned whether all patients included in our study indeed had AD, and did not suffer from vascular dementia or even Creutzfeldt-Jakob disease. Since all our patients were diagnosed according to widely used clinical criteria and had follow-up, this seems unlikely. Furthermore, we found that our results had a dose-response relationship without threshold effect, making it unlikely that they are attributable to a few misdiagnoses. This was also confirmed by an additional analysis, in which we showed that our results, of baseline levels of CSF biomarkers being associated with rate of decline in MMSE, were not attributable to the few cases lacking a typical AD CSF profile.

We show that it is possible to predict the rate of cognitive decline using CSF biomarkers. In the future, these findings might have implications for clinical practice. They could for instance be used in treatment trials, to focus on patients with expected rapid decline. However, further verification is needed. It should also be noted that our patient group is relatively young with patients with a mild degree of dementia, hence data are mainly representative for a memory clinic cohort with the same characteristics. At this time, we think our results mainly have scientific value by adding to the understanding of the pathogenesis of AD.

Aβ$_{1-42}$ is associated with the formation of amyloid, which has been assumed to be an early process in AD. We found that it also predicted, albeit weakly, cognitive decline over time. CSF tau has been suggested to reflects the degree of neuronal degeneration and to be a more general marker for neuronal damage.[15-17;23;24] This seems in agreement with our finding that high tau predicted more rapid cognitive decline, with a high degree of neuronal damage resulting in rapid cognitive decline. In literature it has been assumed that CSF p-tau reflects phosphorylation.[17;25;26] In accordance, phosphorylated tau is considered to be a specific biomarker for AD as it is related to the process of neurofibrillary tangle formation.[25-27] A relatively little increased level of p-tau in combination with a strongly increased level of tau (low p-tau/tau ratio) could be interpreted as a low rate of phosphorylation of tau. We have shown that a low value of the p-tau/tau ratio was the strongest predictor of rapid cognitive decline in AD patients; the predictive value was even stronger than of tau alone. It seems that patients with high rates of neuronal damage, as reflected by high tau, in combination with low phosphorylation, as reflected by relatively less elevated p-tau, have the most aggressive type of AD. Conversely, patients with a high rate of phosphorylation, as evident by levels of p-tau that are proportionally elevated to levels of tau, are prone to a more benign disease course. This seems counterintuitive since high p-tau, as reflector of tangle formation, is associated with core AD pathology. Recent studies found evidence for the fact that tau phosphorylation may help the neurons to escape from an acute apoptotic cell death, while instead leading to neurodegeneration.[28-30] This could imply that the process to avoid apoptosis is in fact the essence of neurodegeneration as seen in AD, fitting the hypothesis that phosphorylation has a compensatory or even protective role.

An alternative explanation for our finding could be that many patients with AD in fact suffer from mixed disease. Post-mortem studies showed mixed types of dementia to be very common.[31;32] AD with concomitant vascular disease has been related to more severe dementia.[33] Also, dementia with Lewy bodies has been associated with rapid cognitive decline, and it is thus conceivable that AD patients with additional alpha-synucleinopathy are at risk of rapid decline.[34] Along this line, our results could indicate that a low p-tau/tau ratio is a reflection of mixed disease of AD, with concomitant cerebrovascular disease or Lewy bodies. However, the fact that co-morbidity in our population was very low, as shown in table 1, seems in contradiction with these thoughts.

Almost all patients were treated with cholinesterase inhibitors at follow-up, but not at baseline. Possibly, patients with a higher p-tau/tau ratio would respond better to cholinesterase inhibitors, which might explain their slower cognitive decline. This is an interesting topic for further study.

Additional studies are needed to unravel the pathophysiological background of our findings. The use of more advanced MRI techniques, measurement of CSF alpha-synucleine and further post-mortem verification of diagnoses could give insight in the extent of the possible role of mixed dementia. Finally, more basic research is required to unravel the role of tau phosphorylation itself.

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CSF biomarkers predict rate of cognitive decline in Alzheimer's disease

3.3

MICROBLEEDS DO NOT AFFECT RATE OF COGNITIVE DECLINE IN ALZHEIMER'S DISEASE

A.E. van der Vlies[1], J.D.C. Goos[1], F. Barkhof[2], P. Scheltens[1], W.M. van der Flier[1,3]

[1] Alzheimer Center & Department of Neurology, Neuroscience Campus Amsterdam,
[2]Department of Radiology, Image Analysis Center and Alzheimer Center,
[3]Department of Epidemiology & Biostatistics, VU University Medical Center,
Amsterdam, the Netherlands.

| ABSTRACT

Objective

To investigate the relationship between brain microbleeds (MBs) and the rate of cognitive decline in Alzheimer's Disease (AD).

Methods

In this cohort study, we studied 221 AD patients with available baseline MRI (1.0 or 1.5T) and at least two Mini Mental State Examinations (MMSE) obtained at least one year apart from our memory clinic. Mean (±standard deviation) follow-up time was 3±1 years and patients had a median of 4 MMSEs (range 2–17). We used linear mixed models with sex and age as covariates to investigate whether MBs influenced the rate of cognitive decline.

Results

Mean age was 68±9, 109 (49%) patients were female and baseline MMSE was 22±4. There were 39 patients (18%) with MBs (median = 2, range 1-27) and 182 without.
Linear mixed models showed that overall, patients declined 2 MMSE points per year. We found no association of the presence of MBs with baseline MMSE or change in MMSE. Adjustment for atrophy, white matter hyperintensities, lacunes and vascular risk factors did not change the results, nor did stratification for MB location, APOEε4 carriership or age-at-onset (≤65 years vs >65 years). Repeating the analyses with number of MBs as predictor rendered similar results.

Conclusion

MBs did not influence the rate of cognitive decline in AD patients. The formerly reported increased risk of mortality in patients with MBs seems not to be attributable to a steeper rate of decline per se, but might be due to vascular events, including (hemorrhagic) stroke.

3.3

| INTRODUCTION

Microbleeds (MBs) are small rounded regions of hypointensities on gradient echo (GRE) T2*-weighted MRI which frequently occur in AD patients.[1-4] Histologically, MBs represent hemosiderin, likely from leakage through cerebral small vessels, contained within surrounding macrophages in the brain parenchyma.[5] In the setting of AD, especially lobar MBs are believed to arise from leakage from fragile amyloid laden vessel walls, defined as cerebral amyloid angiopathy (CAA).[6] The relationship between MBs or CAA and cognition in AD is unclear. Cross-sectionally, some studies find that AD patients with CAA or MBs are more severely cognitively impaired[7;8], while others find no such relationship.[2;3;6;9] Several cross-sectional studies have reported a relationship between MBs and cognition in the elderly with or without increased vascular risk and in patients with small and large vessel disease.[1;10-16] Previous studies have shown that patients with MBs have a higher risk of mortality.[17;18] It is not known, however, whether AD patients with MBs are prone to a more rapid rate of cognitive decline. In the present cohort study, we hypothesized that the presence of MBs reflects a heavier burden of pathology in AD, resulting in a steeper rate of cognitive decline. The aim of this study was to assess the predictive value of baseline MBs on cognitive decline over time in patients with AD.

| METHODS

Patients, design and setting

In this cohort study, we included consecutive patients with AD who presented between 2000 and 2008 at the outpatient memory clinic of the Alzheimer Center of the VU University Medical Center (VUmc), with baseline MRI with GRE sequence on 1.0T or 1.5T and a minimum duration of follow-up of one year. At baseline, all patients underwent a standardized dementia assessment including medical history, informant-based history, physical and neurological examination, laboratory tests, neuropsychological testing, electroencephalogram (EEG) and magnetic resonance imaging (MRI) of the brain. Furthermore, we obtained information on smoking habits, current use of Alzheimer medication anti-thrombotics, and medical history. Hypertension, diabetes mellitus, hypercholesterolemia and myocardial infarction were defined based on self-reported medical history and medication use. Diagnoses of probable AD were made in a multidisciplinary consensus meeting according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) diagnostic criteria.[19] None of the patients had symptomatic brain haemorrhage. Autopsy was available for 5 patients (2 with MBs, 3 without MBs). The diagnosis AD was confirmed in all cases. In one case (no MBs) significant co-existent vascular pathology was mentioned.

We followed patients clinically, with (semi-)annual assessment of their general level of cognitive functioning. The outcome measure was Mini Mental State Examination (MMSE).[20] To be included in this study, patients had to have a minimum of two MMSE's, obtained at least one year apart. The resulting data set included 1021 MMSE scores from 221 patients. Follow-up time varied between one and seven years (mean±sd: 3±1) and patients had a median of 4 MMSE scores (range: 2-17) (table 1).

Table 1. Baseline demographic and clinical characteristics.

	No Microbleeds	≥ 1 Microbleeds
N	182	39
Sex, n (%) female	94 (52)	15 (39)
Age, mean (sd)	67 (9)	71 (8)*
Education, median (range)[a]	5 (2-7)	5 (2-7)
APOE ε4 carriers, n (%)[b]	111 (71)	20 (69)
MMSE at baseline, mean (sd)	22 (4)	22 (4)
Follow-up time (years), mean (sd)	3 (1)	3 (1)
Number of MMSE's, median (range)	4 (2-17)	4 (2-10)
Mortality, n(%)	24 (13%)	8 (21%)
Smoking, n(%)[d]	26 (16%)	3 (8%)
Hypertension, n(%)[a]	48 (27%)	20 (51%)*
Diabetes mellitus, n(%)[a]	12 (7%)	3 (8%)
Hypercholesterolaemia, n(%)[a]	28 (16%)	9 (23%)
Myocardial infarction, n(%)[a]	6 (3%)	3 (8%)
Use of anti-thrombotics, n(%)[a]	32 (18%)	13 (33%)*
Use of Alzheimer medication, n(%)[a]	13 (7%)	2 (5%)
CSF amyloid-beta 1-42, pg/mL[c, e]	459 (166)	406 (153)*
CSF total tau, pg/mL[c, e]	639 (399)	739 (497)
CSF p-tau, pg/mL[c, e]	87 (34)	94 (44)
Microbleeds, median (range)	---	2 (1-27)*
MTA, mean (sd)[e]	1.4 (0.9)	1.9 (1.0)*
GCA, mean (sd)[e]	1 (1)	1 (1)
WMH, mean (sd)[e]	0.8 (0.8)	1.5 (1.0)*
Lacunes, mean (sd)[d]	0 (0)	0 (1)

[a] Data available for 219 patients; [b] Data available for 186 patients; [c] Data available for 158 patients, [d] Data available for 196 patients. [e] Mean (sd) are shown, while analyses were performed with nonparametric Mann-Whitney U tests MMSE = Mini Mental State Examination, p-tau = tau phosphorylized at threonine 181, MTA = medial temporal lobe atrophy, GCA = global cortical atrophy, WMH = white matter hyperintensities. * p <0.05

The study was approved by the local Medical Ethical Committee. All patients gave written informed consent for their clinical data to be used for research purposes.

MRI

Baseline MRI was performed on a 1.0 Tesla machine (n= 179; Magnetom Impact Expert Siemens AG, Erlangen, Germany) or 1.5 Tesla machine (n= 42; Siemens Sonata Syngo, Erlangen, Germany). The scan protocol included T1-weighted, T2-weighted, Flair and GRE images and has been described previously.[1;21] Scan parameters for the axial GRE images used

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for MB detection were as follows: Impact: 19 slices, field of view 250 mm, matrix 256x256, slice thickness: 5 mm, interslice gap: 1 mm, repetition time: 800 ms, echo time: 22 to 25 ms, flip angle 20°. Sonata: 21 slices, field of view 250 mm, slice thickness: 5 mm, interslice gap: 1.5 mm, repetition time: 415 ms, echo time: 25 ms, flip angle 15°.

MRI rating was performed blinded to clinical data. MBs were defined as rounded hypointense homogeneous foci measuring up to 10mm in the brain parenchyma on GRE images. Lesions in sulci possibly representing flow voids from pial vessels and symmetrical lesions in the basal ganglia, supposedly representing iron or calcium deposits, were not taken into account. Hypointensities inside infarcts were not counted as MBs, but regarded to be probable hemorrhagic transformations. Cavernous angiomas were not taken into account. We counted MBs in four lobar regions (frontal, parietal, temporal and occipital) and in two non-lobar regions (basal ganglia including thalamus, and infratentorial). The main determinant was presence of at least one MB. In additional analyses, number of MBs and MB by location were used as determinants.

We performed visual rating of medial temporal lobe atrophy (MTA) on coronal T1-weighted images according to the 5-point (0-4) Scheltens scale.[22] Global cortical atrophy (GCA, range 0-3)[23] and WMH severity (Fazekas, range 0-3)[24] were rated visually on axial FLAIR images; the highest scores represent maximal pathology. We counted lacunar infarcts, defined as well-demarcated lesions from 3 to 15 mm, with a cerebrospinal fluid-like signal on all sequences.

APOE and CSF biomarkers
DNA was isolated from 10 ml EDTA blood. APOE genotype was determined with the Light Cycler APOE mutation detection method (Roche Diagnostics GmbH, Mannheim, Germany). APOE genotype was available for 186 patients. CSF biomarkers were assessed as markers of Alzheimer neuropathology. CSF was obtained by a lumbar puncture. Amyloid β 1-42 ($A\beta_{1-42}$), total tau and tau phosphorylated at threonine-181 (p-tau) were measured by sandwich ELISA (Innotest β-amyloid[1-42], Innotest hTAU-Ag, and Innotest Phosphotau(181P); Innogenetics, Gent, Belgium).[25] CSF was available for 158 patients.

Statistical analysis
We used SPSS 15.0 to perform statistical analyses. Baseline differences between groups were studied using Student's t-test, Mann-Whitney U-test or χ²-test where applicable. We used linear mixed models to assess associations between presence of MBs and the rate of cognitive decline as measured by MMSE. This approach has increased statistical power as it accounts for within-person correlations over time, allows different numbers of assessments, and accounts for varying time intervals between assessments. A random intercept and a random slope with time (in years) were assumed, i.e. baseline MMSE (main effect of MBs) and change in MMSE over time (interaction effect of MBs*time) were allowed to vary between patients. The first model included terms for presence of MBs, time, and the interaction between presence of MBs and time, with sex and age as covariates and

MMSE score as dependent variable. Secondly, we used a model additionally adjusting for MTA, GCA, WMH and presence of lacunes. A third model also adjusted for the following potential confounders: smoking, hypertension, diabetes, hypercholesterolaemia, myocardial infarction, use of anti-thrombotics or Alzheimer medication. Furthermore, we repeated the analyses after stratification according to age-at-onset ($\leq$65 years vs >65 years) and according to APOE ε4 carriership (non-carriers vs carriers). The same models were run with a term for the number instead of the presence of MBs. Finally, we ran the models again to assess the influence of the location of MBs on the rate of MMSE change over time in two ways: first with a categorical variable based on presumed underlying etiology, defined as: 1) no MBs; 2) strictly non-lobar MBs; 3) strictly lobar MBs; 4) both lobar and non-lobar MBs and second with a categorical variable based on laterality: 1) no MBs; 2) left sided MBs; 3) right sided MBs; 4) bilateral MBs.

| RESULTS

Demographic and clinical characteristics of the study sample are presented in table 1. Of the patients, 18% had one or more MBs, and 82% had no MBs. Patients with MBs were older than patients without MBs. Groups did not differ in sex, education, APOEε4 carriership, follow-up time or number of follow-ups. Patients with MBs more often died within the study period, although this difference did not reach significance. Patients with MBs more often had a history of hypertension and they more often used anti-thrombotic medication, but there were no differences in other vascular risk factors. Furthermore, patients with MBs had lower CSF levels of Aβ_{1-42}, but there were no differences in tau or p-tau. Relative to normal values, both groups showed decreased CSF levels of Aβ_{1-42} and increased CSF levels of total tau and p-tau (normal values: Aβ_{1-42} $\leq$ 550, total tau $\geq$ 375, p-tau $\geq$ 52).[25] Patients with MBs had more MTA and WMH than patients without MBs. We found no differences between groups on GCA or number of lacunes.

Linear mixed models with random intercept and slope showed that across groups, patients declined two MMSE points per year (ß (SE) = -2.10 (0.31), p= 0.00). No association of MB presence with baseline MMSE (ß (SE) = 0.26 (0.72), p= 0.72) or rate of cognitive decline (ß (SE) = 0.01 (0.34), p= 0.97) was found (Figure 1). Adjustment for MTA, GCA, WMH and number of lacunes did not change the results (baseline MMSE: ß (SE) = 0.34 (0.76), p= 0.65); rate of decline: ß (SE) = -0.09 (0.34), p= 0.79), nor did further adjustment for smoking, hypertension, diabetes, hypercholesterolaemia, myocardial infarction, use of anti-thrombotics or Alzheimer medication. Also, stratification according to age at onset (Figure 2) and APOE ε4 carriership (Figure 3) did not reveal any associations between MBs and cognition. We repeated all analyses with MBs as a continuous measure to study the associations with the number, rather than the presence, of MBs which did not reveal any associations with baseline MMSE or rate of cognitive decline either. Furthermore, restricting the sample to patients with MBs only showed no associations of number of MBs with baseline MMSE (ß (SE) = -0.15 (0.13), p= 0.23) or rate of cognitive decline (ß (SE) = 0.00 (0.05), p= 0.98).
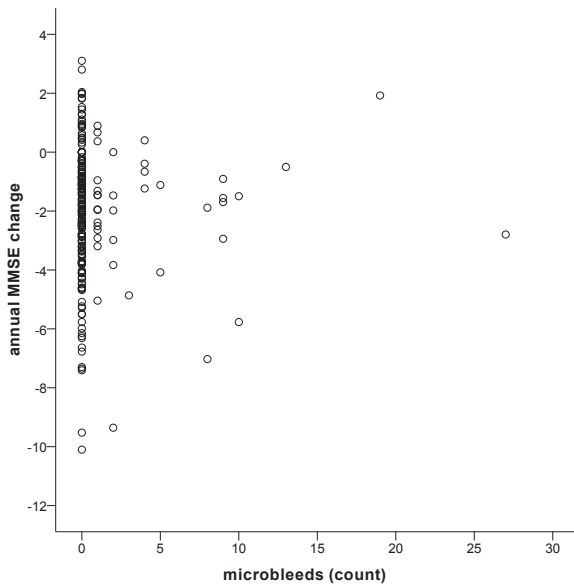
Figure 1. Number of MBs by MMSE change per annum. MMSE change per annum was calculated as last MMSE score minus first MMSE score, divided by follow up time in years. Note that for the statistical analysis linear mixed models were used, which showed no association between MBs and rate of cognitive decline.

Figure 2. Annual MMSE change for patients with and without MBs, stratified according to age at onset. Annual MMSE change was calculated as last MMSE score minus first MMSE score, divided by follow up time in years. Note that for the statistical analysis linear mixed models were used, which showed no association between MBs and rate of cognitive decline for either patients with early or with late disease onset (≤ 65 years or > 65 years).

Figure 3. Annual MMSE change for patients with and without MBs, stratified according to APOE ε4 genotype. Annual MMSE change was calculated as last MMSE score minus first MMSE score, divided by follow up time in years. Note that for the statistical analysis linear mixed models were used, which showed no association between MBs and rate of cognitive decline for either APOE ε4 carriers or non-carriers.
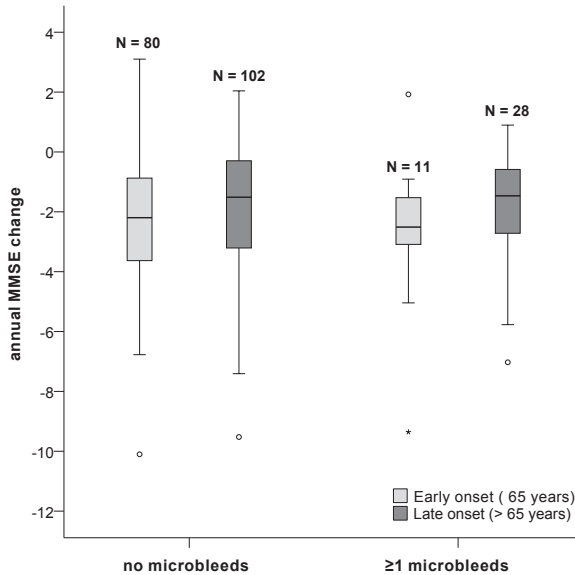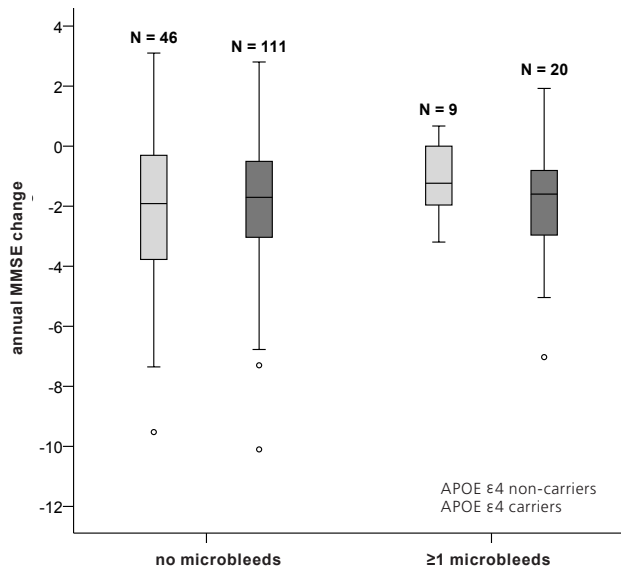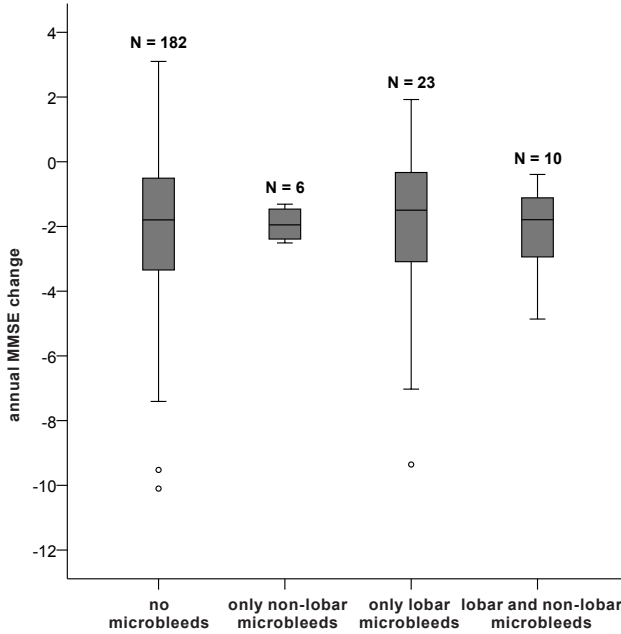

Figure 4. Annual MMSE change according to the presence and location of MBs. Annual MMSE change was calculated as last MMSE score minus first MMSE score, divided by follow up time in years. Note that for the statistical analysis linear mixed models were used, which showed no association between location of MBs and rate of cognitive decline.

When the location of the MBs (no MBs; strictly non-lobar MBs; strictly lobar MBs; both lobar and non-lobar MBs) was taken into account, we observed no association of location of MBs with baseline MMSE or rate of cognitive decline (all *p's* > 0.05) (Figure 4). Similarly, location of MBs in terms of laterality (no MBs; left sided MBs; right sided MBs; bilateral MBs) did not reveal any associations (data not shown).

| DISCUSSION

The main finding of this longitudinal study is that presence and number of MBs are not associated with rate of cognitive decline in AD. Neither stratification for age at onset or APOE-genotype, nor taking into account MB location (deep vs lobar; left hemisphere vs right hemisphere), nor restricting the analysis to patients with MBs only revealed any significant relation between MBs and rate of cognitive decline in this clinical sample of AD patients.

There are two former longitudinal studies that have looked into the relationship between MBs and rate of cognitive decline in Mild Cognitive Impairment (MCI) which are in line with our findings. One study found no difference in MBs between stable and progressive MCI patients after one year.[26] The other study found that the presence of MBs predicts conversion from MCI to dementia, but the signficance of this effect was lost after adjustment for age.[27]

Previous studies have suggested that the presence of one or a few MBs does no real harm, but having multiple MBs is indicative of a more malignant outcome. Despite a modestly - although nonsignficantly - increased mortality rate, we were not able to demonstrate an association between the presence or number of MBs and rate of cognitive decline. In AD, it seems that downstream phenomena such as loss of synapses and neurodegeneration are largely responsible for cognitive decline. Our results suggest that MBs do not affect these downstream pathological Alzheimer processes. Two previous studies showed that patients with multiple MBs have a higher risk of mortality.[17;18] Our current results support the notion that the increased risk of mortality in AD patients with MBs is not related to the Alzheimer process itself, but rather to vascular events, including (hemorrhagic) stroke.

Variability in rate of decline on MMSE in our sample of AD patients was large. The determinants of the rate of decline in AD are largely unknown, as we are presently unable to predict which patients will show faster progression than others. The current study shows that MBs are not an important determinant of rate of decline in AD. We cannot exclude the possibility that MBs have a subtle effect on rate of cognitive decline, but in the context of AD, the clinical significance of such a subtle effect would be limited. Still, MBs may influence change in cognition in other populations, such as nondemented populations. Cross-sectionally, several studies have reported a relationship between MBs and cognition in the elderly with or without increased vascular risk[10-12;14] and in patients with small and large vessel disease.[13;15] Whether MBs also predict change in cognitive performance over time in these populations remains to be determined.

We took into account location of MBs in a number of analyses, but we found no relationship between MBs in a specific location (be it lobar vs non-lobar or left vs right hemisphere) and rate of cognitive decline. It is still conceivable that the localization of MBs could be relevant, but that such an effect is not reflected in the overall MMSE score. Cross-sectional studies have suggested that MBs are largely related to tests of mental speed and executive functioning.[10;13-15] Whether or not the exact location of MBs is relevant in terms of reflecting focal damage remains to be demonstrated however. Alternatively and perhaps more likely is the view that MBs are a tip of the iceberg phenomenon, reflecting widespread underlying vasculopathy.

The current study was designed to relate baseline MRI to change in cognitive functioning. Repeated MRI would have allowed us to relate the incidence of new MBs to rate of cognitive decline, but this was beyond the scope of the current study. In a former study, we have shown that incident MBs occurred in 12% of patients over a two-year period and were not related to change in cognition.[28] Similarly, others have shown that the occurrence of new MBs did not predict clinical decline in patients with intracerebral hemorrhage.[16]

Among the strengths of the current study is the large sample size of patients who were all screened using the same, careful diagnostic work-up. MRI protocols were kept constant over the whole inclusion period. Although misdiagnoses cannot be completely ruled out, all diagnoses were made according to clinical criteria and patients were followed clinically. Moreover, in the majority of patients, CSF biomarkers were available to substantiate the clinical diagnosis. Another strength is the use of linear mixed models for statistical analyses. These models take into account all available data points, allowing patients to have variable numbers of follow-up measurements. In this way, patients with only two available MMSE scores could also be included in the study, as the statistical model appropriately takes into account that the estimate of cognitive decline is less precise in these patients. A potential limitation is the relatively small number of AD patients with MBs, as despite the large sample size of 221 patients with clinical follow up, only 39 had MBs. Still, this number is in agreement with previously reported prevalence of MBs in AD and the large group of patients without any MBs adds power to the stastistical analyses. A second limitation is that although information on the use of Alzheimer medication and other types of medication at baseline was available, use of medication in the course of the disease was not recorded. Nonetheless, although the use of cholinesterase inhibitors and memantine may have influenced the rate of cognitive decline, we do not suspect that this effect would be different for patients with MBs than for those without. Third, our outcome measure was the MMSE, a crude measure of cognitive decline, that does not capture all aspects of disease severity. Still, the MMSE is a generally accepted and widely used test for the evaluation of cognition in elderly patients. A future study should investigate the impact of MBs on the decline of specific cognitive domains and on the relationships between MBs and neuropsychiatry symptoms, also in nondemented populations where subtle effects of MBs may still be discerned.

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Recently, the interest in the clinical consequence of MBs has risen, since amyloid related imaging abnormalities (ARIA) including cerebral MBs have occurred in patients participating in clinical trials with therapeutic agents to lower amyloid-ß burden in AD.[29] In this context, our finding of a lack of association between MBs and the rate of cognitive decline may be of importance. If the rate of cognitive decline -often a primary outcome measure in clinical trials- is not influenced by the presence and number of MBs, excluding patients with MBs may not be necessary. However, it should be noted that the prognosis of ARIA-hemosiderin deposition (ARIA-H) may be different from that of spontaneously occurring MBs. Therefore, further research is needed regarding the risk of accelerated cognitive decline in patients with ARIA-H.

| ACKNOWLEDGEMENTS

3.3

Microbleeds do not affect rate of cognitive decline in Alzheimer's disease

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Microbleeds do not affect rate of cognitive decline in Alzheimer's disease

Ho i crini già grigi,
Ex cathedra parlo

My hair is already grey,
I speak with authority

Don Alfonso, trio *"La mia Dorabella"*
Così fan Tutte
Wolfgang Amadeus Mozart

4

SUMMARY AND GENERAL DISCUSSION

The overall objective of this thesis was to provide insight in cognitive profiles and rate of cognitive decline in AD and to explore which factors are involved in driving the different phenotypes. In the previous chapters these studies were described in detail, focussing on the role of a) APOE genotype, b) CSF biomarkers and c) MRI measures in relation to 1) profiles of neuropsychological impairment, and 2) rate of cognitive decline. In this chapter, the main findings are summarized and relevant issues related to the studies are discussed. The chapter closes with clinical implications of our findings and suggestions for future research.

| SUMMARY

Profiles of neuropsychological impairment in AD

The APOE ε4 genotype increases the risk of AD and has been associated with an earlier age at onset.[1-4] In **chapter 2.1**, we examined whether impairment in specific cognitive domains in AD differed according to APOE genotype and age at onset. Cognitive functions of 229 consecutive AD patients were assessed using a short neuropsychological test battery. APOE ε4 carriers showed more memory impairment than APOE ε4 non-carriers, while in contrast, APOE non-carriers were more severely impaired in naming, mental speed and executive functions. Some of these associations were modified by age at onset, although no main effect for age at onset was found. The APOE effect on memory was most pronounced in early onset homozygous carriers. With regard to naming, older patients performed better as their number of APOE ε4 alleles increased, while younger patients performed worse with increasing number of APOE ε4 alleles. The APOE genotype seems to influence not only the age at onset in sporadic AD patients, but also the cognitive phenotype once patients have reached the stage of dementia.

Chapter 2.2 investigated the relationship between CSF biomarkers and cognitive profiles in AD. We included 177 AD patients who underwent a short neuropsychological assessment. In CSF, levels of $A\beta_{1-42}$, tau and p-tau were measured. K-means cluster analysis was performed with the three biomarkers to obtain 3 clusters of patients. Cluster 1 consisted of 88 patients with relatively high levels of $A\beta_{1-42}$ and low levels of tau and p-tau. Cluster 2 contained 72 patients with relatively low levels of $A\beta_{1-42}$ and high levels of tau and p-tau. Cluster 3 was made up of 17 patients with low levels of $A\beta_{1-42}$ and very high levels of tau and p-tau. No differences between clusters on age, sex, education, APOE genotype, disease duration, functional impairment or disease severity were found. We found that a subgroup of patients with extremely high CSF levels of tau and p-tau showed a distinct cognitive profile with more severe impairment of memory, mental speed and executive functions, which could not be explained by disease severity. In contrast, patients with levels of $A\beta_{1-42}$, tau, and p-tau close to normal showed less impairment of naming and memory abilities than patients with more extreme biomarker levels.

In **Chapter 2.3** we aimed to assess the associations of global atrophy and WMH with neuropsychological function in early and late onset AD. We included 107 patients with sporadic AD (21 early onset and 86 late onset) from our memory clinic. Tests for (working)

memory, language, executive functions, mental speed and attention were administered. Global atrophy and global and lobar WMH were measured using MRI. Linear regression analyses showed that global atrophy was associated with more severely impaired global cognition, working memory, mental speed and executive functions. Significant interactions between global atrophy and age at onset showed that these associations were mostly attributable to patients with early onset AD. By contrast, an association between global atrophy and memory was found, which was specifically attributable to late onset AD patients. No associations between global WMH and cognitive function were found, but analyses of regional WMH showed that temporal WMH was associated with impaired memory, and frontal WMH with slower mental speed. We concluded that cortical atrophy, a key feature of AD, is linked to a wide range of cognitive functions, specifically in early onset AD patients. WMH was not influenced by age at onset, indicating that, if WMH are present in early onset patients, they have a similar effect on cognition as in older patients.

Rate of cognitive decline in AD

Our hypothesis that APOE genotype is involved with more than the risk of developing AD and the age at onset alone was supported by our findings in **chapter 3.1**. In this longitudinal study, we aimed to compare the rate of cognitive decline in patients with early and late onset AD and to investigate the potentially modifying influence of APOE genotype. We included 99 patients with early onset AD and 192 patients with late onset AD who had at least 2 MMSE scores (range 2-14) obtained at least one year apart. Linear mixed models were performed to investigate the rate of cognitive decline dependent on age at onset and APOE genotype. Age at onset was not associated with baseline MMSE. However, with 2.4 points decline per year, patients with early onset showed faster decline than patients with late onset (-1.7 points/year). After stratification according to APOE genotype, APOE ε4 non-carriers with early onset showed faster cognitive decline than non-carriers with late onset (-2.4 vs -1.3 points/year). In APOE ε4 carriers, no difference in rate of cognitive decline was found between patients with early and late onset. We concluded that patients with early onset AD show more rapid cognitive decline than patients with late onset, suggesting that early onset AD follows a more aggressive course. This effect seems to be most prominent in patients with early onset that do not carry the genetic APOE ε4 risk factor for AD.

In **chapter 3.2**, the relationship between CSF biomarkers and cognitive decline was studied using a longitudinal design. We hypothesized that high tau, especially in combination with relatively low p-tau, is a marker of rapid decline, since it has been associated with fast neuronal degeneration. We included 151 AD patients, of whom we had baseline CSF, from our memory clinic. All patients had at least two MMSE scores, obtained no less than one year apart. Mean follow-up period was 2 years. Linear mixed models were used to assess associations between CSF biomarkers and the rate of cognitive decline as measured with the MMSE. No relations between any CSF biomarker and baseline MMSE were found. However, CSF biomarkers did predict cognitive decline over time. A low p-tau/tau-ratio

was the strongest predictor with a dose dependent effect (lowest vs highest quintile: 2.9 vs 1.3 MMSE-points annual decline), indicating that a combination of high CSF tau without proportionally elevated p-tau is associated with a faster rate of cognitive decline. In addition, low Aß$_{1-42}$, high tau and high tau/ Aß$_{1-42}$-ratio were also associated with rapid cognitive decline.

In **chapter 3.3** we investigated the relationship between brain microbleeds and the rate of cognitive decline in AD. We studied 221 AD patients with available baseline MRI (1.0 or 1.5T) and at least two MMSE scores obtained at least one year apart from our memory clinic. Mean follow-up duration was 3 years and patients had a median of 4 MMSEs. There were 39 patients with microbleeds (median = 2, range 1-27) and 182 without. Linear mixed models showed that overall, patients declined 2 MMSE points per year. We found no relation between the presence of microbleeds and baseline MMSE or change in MMSE over time. Adjustment for atrophy, WMH, lacunes and vascular risk factors did not change the results, nor did stratification according to microbleed location, APOE ε4 carriership or age at onset (≤65 years vs >65 years). Repeating the analyses with number of microbleeds as predictor rendered similar results. We concluded that microbleeds do not influence the rate of cognitive decline in AD patients. The formerly reported increased risk of mortality in patients with microbleeds seems not to be attributable to a steeper rate of decline per se, but might be due to vascular events, including (hemorrhagic) stroke.

| METHODOLOGICAL ISSUES

Selection of patient population

This thesis has a unique approach to studying cognition in AD. Rather than comparing AD patients to healthy controls, we have studied the cognitive spectrum within the disease itself, acknowledging that not all AD patients are the same, but that cognitive, biological and pathophysiological factors vary greatly amongst AD patients. Studying risk factors such as age at onset and APOE ε4 genotype in relation to variability in clinical manifestations of AD, as opposed to comparing AD patients to healthy elderly, has lead to increased understanding of the biological factors underlying this phenotypical heterogeneity.

Since the diagnosis of early onset dementia is a point of great interest to the VUmc Alzheimer Center, the group of early onset AD patients is overrepresented in the cohort, offering unique potential: younger AD patients suffer from a more pure form of the disease, which makes them an ideal model to study the pathogenesis of AD. Furthermore, heterogeneity in manifestation is especially evident within the group of early onset AD.

Several factors may have led to a biased selection of patients for our studies. With regard to the studies of neuropsychological profiles in AD, in two studies (Chapters 2.2 and 2.3) we have only used patients with complete neuropsychological data. Since patients that are more severely cognitively impaired are often unable to complete all neuropsychological tests and

Summary and general discussion

therefore have missing data on one or more tests, this may have led to a bias in our results. We cannot rule out that these patients may have a different cognitive profile than patients who were able to complete all tests, although we have no indication that more severely cognitively impaired patients would have a distinctly different cognitive profile. Rather, as patients become more severely demented, more cognitive domains become impaired, making it harder to distinguish cognitive profiles and even to differentiate between the various types of dementia. Therefore, it seems to be a strength that we have only included patients who were mildly demented.

The studies of rate of cognitive decline may have been subject to survivor bias: patients with extremely fast decline, severe disability or short survival, which prevents them from returning to the memory clinic, may have been lost to follow-up. This may have resulted in a possible underestimation of the rate of decline of all or some of the reported subgroups. This is especially relevant for the microbleeds study, in which we found no effect on cognitive decline, while previous cross-sectional studies have reported a relationship between microbleeds and cognition,[5-9] and between microbleeds and risk of mortality.[10;11] However, it should be noted that there are other, cross-sectional, studies that also found no relationship between microbleeds and cognition.[12-14]

Neuropsychological assessment
The studies in Chapter 2 are based on a standard test battery that screens several cognitive domains. This gives more detailed information than the use of cognitive screening tests such as the MMSE or the CAMCOG alone. Since this battery is used for every patient in our memory clinic, we have been able to study specific cognitive domains in large patient samples, adding to the reliability of the results.

The test battery lacks a cognitive test specifically designed to assess praxis and visuospatial functions. An adequate measure for praxis and visuospatial functions is hard to find and therefore most studies do not include such a test. This is particularly relevant for AD studies, since often these domains are among the first to be impaired in non-amnestic AD patients, while by contrast they may be preserved for a relatively long time in typical, amnestic AD patients. It would therefore be advisable to include tests for praxis and visuospatial functions in the standard test battery. In the last few years, three subtests of the Visual Object and Space Perception Battery (VOSP) have been added to the test battery to assess visuospatial functions.[15] In a recent study, our lab showed that patients with early onset AD performed worse on several non-memory domains, amongst which visuospatial functions, than late onset AD patients.[16] Lately, van Heugten's test for apraxia[17] has been added to the test battery and data on praxis abilities in our cohort are now being collected.

For the studies in Chapter 3, we have used the MMSE as a measure for cognitive decline. Although the MMSE is only a short cognitive screening test and a rather crude cognitive measure, it is a generally accepted - and in our experience a robust- measure for cognitive

decline.[18] A large advantage of the MMSE is that it is easy to administer, which has enabled us to collect data from a large number of patients over a long period of time, even measured at up to 17 time points. A possible disadvantage is that the MMSE only allows us to study global cognitive decline, whereas it would also be interesting to study decline in different cognitive domains. Since a few years, we have started to subject patients to a neuropsychological assessment at multiple time points. However, we need to be aware that as this is more straining on the patients, the risk of survivor bias will increase. Therefore, studies of global cognitive decline as reported in this thesis, together with studies of decline in specific cognitive domains that are currently going on, will give a more complete picture of the mechanisms underlying decline in AD.

| CLINICAL IMPLICATIONS

Among the general public, but also among primary care physicians, a stereotypical picture of AD still exists. It is the classical picture of an older person with predominantly memory problems, which slowly get worse, followed by the impairment of other cognitive functions, until finally the patient can no longer take care of him/herself, is admitted to a nursing home and dies. This thesis has shown that this general idea should be adjusted. Many AD patients suffer from impairment in other cognitive domains, such as language, mental speed and/or executive functions, while their memory function is relatively spared in the early stages.

We were able to link genetic, imaging and pathophysiological substrates to cognitive subtypes of AD. These findings may have important clinical implications. In recent years, more and more effort has been directed towards the development of therapies for AD. The heterogeneous nature of AD suggests that it is not very likely that a single therapy ("magic bullet") will benefit all AD patients. In a future of "personalized medicine", it is increasingly important to be able to identify meaningful subtypes, which all will need a different approach. Our results suggest that variability in clinical manifestation in AD is related to biological substrates, which may point at specific pathways. It is conceivable that combining cognitive profile and biological markers will be helpful in the tailor-made allotment of future therapies.

Many drug trials use cognitive change as their primary outcome measure in testing the efficacy of the drug in question. This thesis has shown that age at onset, APOE genotype, and CSF biomarkers are all related to the rate of cognitive decline in AD. It is advisable for designers of drug trials to take these factors into account when evaluating new interventions. As one example, the interest in the clinical consequences of microbleeds has recently grown, since amyloid related imaging abnormalities (ARIA) including hemosiderin deposition (ARIA-H) have occurred in patients participating in clinical trials with therapeutic agents to lower amyloid-ß burden in AD.[19] In this context, our finding of a lack of association between microbleeds (as one form of ARIA-H) and the rate of cognitive decline may be of importance. If the rate of cognitive decline is not influenced by the presence and number

of microbleeds, excluding patients with microbleeds may not be necessary for that reason. However, it should be noted that the prognosis of ARIA-H may be different from that of spontaneously occurring microbleeds. Therefore, further research is needed regarding the risk of accelerated cognitive decline in patients with ARIA-H.

| RECOMMENDATIONS FOR FUTURE RESEARCH

In the literature, several attempts to define subtypes in different modalities (e.g. cognition, MRI, CSF) have been made.[20-25] In this thesis, we have categorized patients based on biological and pathophysiological factors and have studied whether this resulted in distinct neuropsychological profiles. The next step is to look at the data from the opposite direction, by first determining which cognitive clusters of AD patients can be found. Clustering of cognitive data should be done in a data driven manner, without a priori hypotheses with regard to the number or type of subpopulations, and the results should be validated in another sample to test their generalizability. Subsequently, using a more holistic approach, efforts should be made to relate the subtypes to the combination of biological and pathophysiological factors such as APOE genotype, CSF biomarkers, EEG abnormalities, MRI-measures and age at onset.

Furthermore, besides studying the relationships between cognitive phenotypes and known biological and pathophysiological factors, major efforts should be put in the quest for new biomarkers in blood, CSF or other fluids. Biomarkers are needed to predict disease progression (prognostic markers) and to predict response to therapy (theragnostic markers). Biological factors, which show meaningful variation within the spectrum of AD, are likely candidates for these purposes. For example, it is generally accepted that other genetic factors beside APOE genotype influence the pathogenesis of sporadic AD. Genomewide association studies to date have chiefly focused on identifying disease susceptibility factors.[26] The next step is -in stead of comparing the DNA of AD patients to the DNA of controls- to relate the DNA of different cognitive subtypes of AD with each other in an endeavour to identify disease modifying factors.

Following on the idea of integrating data on cognitive subtypes with biological, genetic and pathophysiological factors in a holistic approach, this could be extended to the rate of cognitive decline. Following up on the studies in this thesis that have looked at the relationship between individual factors such as APOE genotype, CSF biomarkers and age at onset, it would be interesting to develop an algorithm based on these and other biological, genetical and pathophysiological factors that can predict the rate of cognitive decline of individual AD patients.

Machine learning algorithms have recently been proposed to combine multiple AD features derived from brain imaging and other biomarkers, for Mild Cognitive Impairment (MCI) and AD classification and for predicting conversion from MCI to AD.[27] Several studies have performed diagnostic classification based on several MRI measures.[28;29] Others have

extended on this by adding data from other modalities, such as incorporating demographic variables such as age, sex, and APOE genotype and including CSF biomarkers[30], and PET data.[27;31;32] The next step would be to combine data of different modalities in order to predict the rate of cognitive decline once the patient has developed dementia. Besides the obvious benefit for the patient and his/her caretakers of being able to predict the rate of cognitive decline, it may provide vital information for designing, powering, and implementing future clinical trials.

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Qui la celeste parca	The time has not yet come
non tronca ancor lo stame alla mia vita!	for the hand of fate to end my wretched life!
Ma dove andrò? e chi mi porge aita?	Where can I turn? What friend have I to help me?
Ove son le mie schiere? Ove son le legioni,	What is left of my army? My invincible legions,
che a tante mie vittorie il varco apriro?	which so often in the past have brought me victory?
Solo in queste erme arene	Vanquished, and alone in this desert,
al monarca del mondo errar conviene?	must the master of an empire defenceless wander?

Cesare, recit and aria *"Dall'ondoso periglio…Aure, deh, per pietà"*
Giulio Cesare in Egitto
Georg Friedrich Händel

5

SAMENVATTING

| COGNITIEVE PROFIELEN IN DE ZIEKTE VAN ALZHEIMER
HET HERKENNEN VAN HAAR VELE GEZICHTEN

In dit hoofdstuk worden de in dit proefschrift beschreven onderzoeken samengevat. Na de samenvatting van de beschreven studies volgen een bespreking van de implicaties en suggesties voor verder onderzoek.

Dit proefschrift gaat uit van de observatie dat niet alle Alzheimerpatiënten aan elkaar gelijk zijn. Veel patiënten met de ziekte van Alzheimer (ZvA) presenteren zich met het klassieke beeld van stoornissen in het geheugen. Er zijn echter ook patiënten waarbij stoornissen in bijvoorbeeld de taal of de uitvoerende functies meer op de voorgrond staan dan stoornissen in het geheugen. Er is niet veel bekend over de biologische factoren die aan het spectrum van cognitieve stoornissen binnen de ZvA ten grondslag liggen. Bovendien worden patiënten met een atypische presentatie van de ziekte vaak gedurende jaren verkeerd gediagnosticeerd voordat uiteindelijk de diagnose ZvA gesteld wordt. Een diepgaander begrip van de verschillende subtypes binnen de ZvA, hun pathologie en de factoren die ten grondslag liggen aan de differentiatie van fenotypen is dus van essentieel belang voor een bredere erkenning van de vele gezichten van de ZvA. Bovendien wordt er hard aan gewerkt om therapeutische interventies te ontwikkelen die de progressie van de ziekte vertragen of zelfs tot stilstand brengen. Het erkennen van subtypes van de ZvA zal waarschijnlijk een belangrijke rol spelen in de ontwikkeling en toewijzing van (toekomstige) behandelingen op maat.

Het doel van dit proefschrift is om inzicht te verschaffen in cognitieve profielen en de snelheid van cognitieve achteruitgang binnen de ZvA en te onderzoeken welke factoren betrokken zijn bij de verschillende fenotypen. Het proefschrift richt zich op twee aspecten van klinische presentatie bij Alzheimerpatiënten:
• Profielen van neuropsychologische stoornissen
• Snelheid van cognitieve achteruitgang

We onderzochten de mate waarin a) het APOE genotype, b) biomarkers in het hersenvocht and c) MRI bevindingen de verschillen in cognitieve profielen en snelheid van cognitieve achteruitgang tussen patiënten konden verklaren. Alle studies werden uitgevoerd met Alzheimerpatiënten (in tegenstelling tot vergelijkingen met andere patiëntgroepen of gezonde ouderen) om verschillende subgroepen binnen dezelfde ziekte te verkennen.

| SAMENVATTING VAN DE STUDIES

Profielen van neuropsychologische stoornissen

Het APOE ε4 genotype verhoogt het risico op de ZvA en is in verband gebracht met de aanvang van de ziekte op jongere leeftijd.[1-4] In **hoofdstuk 2.1** onderzochten we of patronen van stoornissen in specifieke domeinen binnen de ZvA samen hingen met verschillen in APOE genotype en de leeftijd waarop de ziekte aanving (voor of na het 65[e] levensjaar). De cognitieve functies van 229 Alzheimerpatiënten werden onderzocht met behulp van een korte

Samenvatting

neuropsychologische testbatterij. APOE ε4 gendragers hadden meer geheugenstoornissen dan niet-gendragers, terwijl niet-gendragers meer stoornissen in het benoemen, de mentale snelheid en de uitvoerende functies lieten zien. Sommige van deze verbanden werden gemodificeerd door de aanvangsleeftijd, alhoewel hiervoor geen hoofdeffect werd gevonden. Het APOE effect op geheugen was het meest uitgesproken in jonge homozygote gendragers. Oude patiënten presteerden beter op een benoemtaak naarmate ze meer APOE ε4 allelen hadden, terwijl jonge patiënten hierop slechter presteerden naarmate ze meer APOE ε4 allelen hadden. Concluderend lijkt het APOE genotype niet alleen het risico op het krijgen van de ziekte te beïnvloeden, maar ook het cognitieve fenotype wanneer patiënten eenmaal het dementiestadium hebben bereikt.

In het hersenvocht (cerebrospinal fluid, CSF) kunnen eiwitten gemeten worden die de neuropathologie van de ZvA weerspiegelen. Bij Alzheimerpatiënten is het gehalte van het eiwit $A\beta_{1-42}$ lager dan bij gezonde mensen en zijn de gehalten tau en p-tau hoger dan bij gezonde mensen. **Hoofdstuk 2.2** onderzocht het verband tussen deze zogeheten biomarkers en cognitieve profielen binnen de ZvA. We includeerden 177 Alzheimerpatiënten die allen een kort neuropsychologisch testonderzoek hadden ondergaan. In het hersenvocht werden de concentraties $A\beta_{1-42}$, tau en p-tau gemeten. De drie biomarkers werden gebruikt om met behulp van K-means cluster analyse een drietal clusters van patiënten te vormen. Cluster 1 bestond uit 88 patiënten met relatief hoge $A\beta_{1-42}$ gehalten en lage tau en p-tau gehalten. Cluster 2 omhelsde 72 patiënten met relatief lage $A\beta_{1-42}$ concentraties en hoge tau en p-tau concentraties. Cluster 3 bestond uit 17 patiënten met lage $A\beta_{1-42}$ gehalten en zeer hoge tau en p-tau gehalten. Leeftijd, geslacht, opleidingsniveau, APOE genotype, ziekteduur, functionele beperking en ziekte-ernst verschilden niet tussen de verschillende clusters. Cluster 3, de groep met extreem hoge gehalten tau en p-tau, vertoonde een specifiek cognitief profiel met ernstigere stoornissen op het gebied van geheugen, mentale snelheid en uitvoerende functies dan bij de andere clusters. Dit effect kon niet verklaard worden door een verschil in ziekte-ernst. Patiënten met $A\beta_{1-42}$, tau en p-tau concentraties die vrij dicht bij de normale waarden lagen (cluster 1) daarentegen, vertoonden minder beperkingen van benoem- en geheugencapaciteiten dan patiënten met extremere biomarkerconcentraties.

De MRI-scans van de hersenen van Alzheimerpatiënten vertonen meestal cerebrale atrofie; afsterving van hersencellen die vaak begint in de mediale temporaalkwab, zich uitbreidt naar de rest van de cortex en uiteindelijk resulteert in globale atrofie. Bovendien zijn wittestofafwijkingen (WSA), vermoedelijk veroorzaakt door aantasting van de kleine bloedvaten, vaker zichtbaar op de MRI-scans van Alzheimerpatiënten dan op die van gezonde ouderen. In **hoofdstuk 2.3** onderzochten we de verbanden tussen globale atrofie en WSA enerzijds en neuropsychologisch functioneren anderzijds bij jonge en oude Alzheimerpatiënten. Uit onze geheugenpolikliniek werden 107 patiënten met de ZvA (21 jonge en 86 oude patiënten) geïncludeerd. Testen voor (werk)geheugen, taal, uitvoerende functies, mentale snelheid en aandacht waren afgenomen. Globale atrofie en globale en regionale WSA werden gemeten met behulp van MRI. Lineaire regressie analyses lieten zien

dat meer globale atrofie samenhing met ernstigere beperkingen van de globale cognitie, het werkgeheugen, de mentale snelheid en de uitvoerende functies. Interacties tussen globale atrofie en aanvangsleeftijd lieten zien dat deze verbanden vooral te zien waren bij jonge Alzheimerpatiënten. Hiermee contrasterend werd een verband tussen globale atrofie en geheugen gevonden dat specifiek van toepassing was op oude Alzheimerpatiënten. Er werden geen verbanden tussen globale WSA en cognitief functioneren gevonden, maar analyse van regionale WSA vertoonden een verband tussen temporale WSA en gestoord geheugen en tussen frontale WSA en mentale traagheid. We concludeerden dat corticale atrofie, een hoofdkenmerk van de ZvA, specifiek bij jonge Alzheimerpatiënten verbonden is aan diverse cognitieve functies. WSA hingen niet samen met de aanvangsleeftijd, wat impliceert dat als jonge Alzheimerpatiënten WSA hebben, dit eenzelfde effect op de cognitie heeft als bij oude Alzheimerpatiënten.

Snelheid van cognitieve achteruitgang

De veronderstelling dat het APOE genotype betrokken is bij meer dan alleen het risico op de ZvA en de aanvangsleeftijd, werd bevestigd door de bevindingen die beschreven staan in **hoofdstuk 3.1**. In deze longitudinale studie vergeleken we de snelheid van cognitieve achteruitgang van jonge Alzheimerpatiënten met die van oude Alzheimerpatiënten en onderzochten we in hoeverre het APOE genotype hierop van invloed is. We includeerden 99 Alzheimerpatiënten die de ziekte voor hun 65[e] levensjaar hadden gekregen en 192 patiënten die de ziekte op latere leeftijd ontwikkelden. Als uitkomstmaat gebruikten we de Mini Mental State Examination (MMSE), een korte, globale test voor de ernst van de dementie. Alle patiënten hadden minstens 2 MMSE-scores (range 2-14), waarbij er minstens een jaar tussen het verkrijgen van de scores zat. We gebruikten linear mixed models om de snelheid van cognitieve achteruitgang afhankelijk van aanvangsleeftijd en APOE genotype te onderzoeken. Aanvangsleeftijd hing niet samen met de uitgangsmeting. Echter, met 2,4 punten achteruitgang per jaar, vertoonden jonge patiënten een snellere achteruitgang dan oude patiënten (1,7 punten/jaar). Na stratificatie op basis van APOE genotype, vertoonden jonge APOE ε4 niet-gendragers snellere cognitieve achteruitgang dan oude niet-gendragers (2,4 vs 1,3 punten/jaar). Er was geen verschil in de snelheid van cognitieve achteruitgang tussen jonge en oude APOE ε4 gendragers. We concludeerden dat jonge Alzheimerpatiënten sneller cognitief achteruit gingen dan patiënten met een late aanvangsleeftijd, wat suggereert dat de ZvA vaak een agressiever beloop heeft wanneer je hem op jongere leeftijd krijgt. Dit effect lijkt het sterkst bij jonge Alzheimerpatiënten die geen drager zijn van de genetische risicofactor APOE ε4.

In **hoofdstuk 3.2** bestudeerden we het verband tussen CSF biomarkers en de snelheid van cognitieve achteruitgang in een longitudinale studie. We veronderstelden dat een hoge concentratie tau, vooral in combinatie met een lage concentratie p-tau, een marker is van snelle achteruitgang, aangezien dit geassocieerd is met snelle neuronale degeneratie. We includeerden 151 Alzheimerpatiënten, waarvan we bij de uitgangsmeting CSF hadden verzameld, uit onze geheugenpolikliniek. Alle patiënten hadden minstens 2 MMSE-scores,

waarbij er ten minste een jaar tussen de scores zat, en werden gemiddeld gedurende 2 jaar gevolgd. We gebruikten linear mixed models om verbanden tussen CSF biomarkers en snelheid van cognitieve achteruitgang, zoals gemeten met de MMSE, te onderzoeken. Er werden geen verbanden gevonden tussen één van de CSF biomarkers en MMSE op de uitgangsmeting. CSF biomarkers voorspelden echter wel cognitieve achteruitgang over de tijd. Een lage waarde van de verhouding tussen p-tau en tau was de sterkste voorspeller, met een dosisafhankelijk effect (laagste vs hoogste kwintiel: 2,9 vs 1,3 punten/ jaar), wat aangeeft dat een combinatie van een hoge concentratie CSF tau zónder een proportioneel verhoogd p-tau samenhangt met een snellere cognitieve achteruitgang. Bovendien hingen laag $A\beta_{1-42}$, hoog tau en een hoge tau/$A\beta_{1-42}$-ratio ook samen met snelle cognitieve achteruitgang.

In **hoofdstuk 3.3** onderzochten we het verband tussen microbloedingen in de hersenen en de snelheid van cognitieve achteruitgang in de ZvA. We onderzochten 221 Alzheimerpatiënten van onze geheugenpolikliniek met een MRI bij de uitgangsmeting en minstens 2 MMSE-scores, waarbij er ten minste een jaar tussen het verkrijgen van de scores zat. Patiënten werden gemiddeld 3 jaar gevolgd en hadden een mediaan van 4 MMSE scores. Er waren 39 patiënten met microbloedingen en 182 patiënten zonder. Linear mixed models lieten zien dat de hele groep patiënten gemiddeld 2 punten per jaar achteruitging op de MMSE. We vonden geen verband tussen de aanwezigheid van microbloedingen en de MMSE bij de uitgangsmeting of de verandering in MMSE over de tijd. Correcties voor atrofie, WSA, lacunes en vasculaire risicofactoren lieten eveneens geen effect zien, evenmin als stratificatie op basis van de lokalisatie van de microbloedingen, APOE ε4 genotype of aanvangsleeftijd. Het herhalen van de analyses met het aantal microbloedingen leverde vergelijkbare resultaten op. We concludeerden dat microbloedingen niet van invloed zijn op de snelheid van cognitieve achteruitgang in Alzheimerpatiënten. Het eerder gerapporteerde verhoogde risico op mortaliteit bij patiënten met microbloedingen lijkt niet te wijten aan een snellere achteruitgang, maar zou veroorzaakt kunnen worden door vasculaire incidenten, waaronder hersenbloedingen.

| METHODOLOGISCHE OVERWEGINGEN

Selectie van de onderzoekspopulatie

Dit proefschrift benadert het bestuderen van cognitie in de ZvA op een unieke wijze. In plaats van het bestuderen van Alzheimerpatiënten in vergelijking met gezonde ouderen, hebben we het cognitieve spectrum binnen de ziekte zelf onderzocht. Op deze manier wordt recht gedaan aan de observatie dat niet alle Alzheimerpatiënten hetzelfde zijn, maar dat cognitieve, biologische en pathofysiologische factoren sterk verschillen tussen Alzheimerpatiënten. Het bestuderen van risicofactoren zoals aanvangsleeftijd en APOE ε4 genotype in verhouding tot variabiliteit in klinische manifestaties in de ZvA - in tegenstelling tot het vergelijken van Alzheimerpatiënten met gezonde ouderen - heeft geleid tot een

grondiger begrip van de biologische factoren die aan deze fenotypische heterogeniteit ten grondslag liggen.

Aangezien de diagnostiek van dementie op jonge leeftijd een speerpunt is van het VUmc Alzheimercentrum, is de groep Alzheimerpatiënten met een vroege aanvangsleeftijd oververtegenwoordigd in het cohort, wat een unieke kans biedt: jonge Alzheimerpatiënten lijden aan een meer pure vorm van de ziekte, wat hen tot een ideaal model maakt om de pathogenese van de ZvA te bestuderen. Bovendien is met name binnen de groep met jonge Alzheimerpatiënten heterogeniteit zichtbaar.

Er is een aantal factoren dat mogelijk geleid heeft tot een bias in de patiëntenselectie van onze studies. Met betrekking tot de onderzoeken naar neuropsychologische profielen in de ZvA, hebben we in twee studies (hoofdstukken 2.2 en 2.3) alleen gegevens gebruikt van patiënten waarvan de neuropsychologische data compleet waren. Aangezien patiënten die ernstigere cognitieve stoornissen hebben vaak niet in staat zijn om alle neuropsychologische tests te voltooien en daarom uitgesloten zijn van de studies, kan dit geleid hebben tot een vertekend beeld. We kunnen niet uitsluiten dat deze patiënten een ander cognitief profiel hebben dan patiënten die wel in staat waren om alle tests te maken, hoewel we daarvoor geen aanwijzingen hebben. Integendeel, naarmate patiënten ernstiger dement worden, raken meer cognitieve domeinen gestoord, wat het moeilijker maakt om cognitieve profielen te onderscheiden en zelfs om de verschillende typen dementie van elkaar te onderscheiden. Het lijkt daarom vooral een sterk punt dat we alleen gegevens van patiënten die mild dementerend waren hebben gebruikt.

De studies naar de snelheid van cognitieve achteruitgang hebben mogelijk geleden onder survivor bias: patiënten met extreem snelle achteruitgang, ernstige beperkingen of korte overlevingsduur welke hen beletten om nog naar de geheugenpolikliniek te komen, hebben daardoor geen vervolgmeting gekregen en zijn dus niet in de studies opgenomen. Mogelijk heeft dit geresulteerd in een onderschatting van de snelheid van sommige of van alle gerapporteerde subgroepen. Dit is in het bijzonder relevant voor het onderzoek met betrekking tot microbloedingen, waarin we geen effect vonden op snelheid van cognitieve achteruitgang, terwijl eerdere cross-sectionele studies een verband tussen microbloedingen en cognitie[5-9] en tussen microbloedingen en het risico op mortaliteit[10;11] beschreven. Het moet echter opgemerkt worden dat er andere, cross-sectionele studies zijn die eveneens geen verband vonden tussen microbloedingen en cognitie.[12-14]

Neuropsychologisch onderzoek

De onderzoeken in hoofdstuk 2 zijn gebaseerd op een standaard testbatterij die een aantal cognitieve domeinen onderzoekt. Dit geeft meer gedetailleerde informatie dan enkel het gebruik van een cognitieve screeningstest zoals de MMSE of de CAMCOG. Aangezien deze batterij gebruikt wordt voor elke patiënt in onze geheugenpolikliniek, waren we in staat om specifieke cognitieve domeinen in grote patiëntgroepen te bestuderen, wat bijdraagt aan de betrouwbaarheid van de resultaten.

Aan de testbatterij ontbreekt een cognitieve test die speciaal ontwikkeld is om de praxis en de visuospatiële functies te onderzoeken. Het is moeilijk om een passende test voor deze functies te vinden en daarom wordt zo'n test in weinig studies gebruikt. Dit is echter juist relevant in het kader van de ZvA, aangezien deze domeinen vaak als één van de eersten worden aangetast in niet-amnestische Alzheimerpatiënten, terwijl ze vaak relatief lang gespaard blijven in typische, amnestische Alzheimerpatiënten. Het is daarom aan te raden om tests voor praxis en visuospatiële functies op te nemen in de testbatterij. In de laatste paar jaar zijn drie subtests van de Visual Object and Space Perception Battery (VOSP) toegevoegd aan de testbatterij om de visuospatiële functies te onderzoeken.[15] In een recente studie heeft onze onderzoeksgroep aangetoond dat jonge Alzheimerpatiënten slechter presteerden op verschillende niet-geheugen domeinen, waaronder visuospatiële functies, dan oude Alzheimerpatiënten.[16] Onlangs is ook Van Heugten's apraxietest[17] aan de testbatterij toegevoegd en data op het gebied van praxis worden nu in ons cohort verzameld.

Voor de onderzoeken in hoofdstuk 3 hebben we de MMSE gebruikt als maat voor cognitieve achteruitgang. Hoewel de MMSE slechts een korte cognitieve screeningstest is en een vrij grove maat vormt, is hij algemeen geaccepteerd en is het in onze ervaring een robuuste maat voor cognitieve achteruitgang.[18] Een groot voordeel van de MMSE is dat hij makkelijk af te nemen is, wat ons in staat heeft gesteld om data te verzamelen van een groot aantal patiënten gedurende een lange periode, tot zelfs 17 meetpunten. Een nadeel van de MMSE is dat hij ons alleen in staat stelt om globale cognitieve achteruitgang te meten, terwijl het ook interessant zou zijn om de achteruitgang van verschillende cognitieve domeinen te onderzoeken. Sinds een aantal jaren krijgen onze patiënten herhaalde neuropsychologische onderzoeken over de tijd. Echter, naarmate dit zwaarder wordt voor de patiënten, wordt ook het risico op survivor bias verhoogd. De studies naar globale cognitieve achteruitgang zoals beschreven in dit proefschrift, in combinatie met de studies naar achteruitgang in specifieke cognitieve domeinen die momenteel uitgevoerd worden, zullen een meer compleet beeld geven van de mechanismen die ten grondslag liggen aan cognitieve achteruitgang in de ZvA.

Klinische implicaties

Onder het grote publiek, maar ook onder artsen die werken in de eerstelijn, bestaat nog steeds een stereotype beeld van hoe de ZvA eruit ziet. Het is het klassieke beeld van een ouder iemand met voornamelijk geheugenproblemen, die langzamerhand groter worden, gevolgd door de aftakeling van andere cognitieve functies waarna uiteindelijk de patiënt niet meer voor zichzelf kan zorgen, opgenomen wordt in een verpleeghuis en komt te overlijden. Dit proefschrift heeft aangetoond dat dit algemene idee moet worden bijgesteld. Veel Alzheimerpatiënten lijden aan stoornissen in andere cognitieve domeinen, zoals taal, mentale snelheid en/of uitvoerende functies, terwijl hun geheugen *relatief* gespaard is in de vroege stadia van de ziekte.

Wij hebben genetische, MRI en pathofysiologische substraten aan cognitieve subtypes van de ZvA kunnen verbinden. Deze bevindingen kunnen belangrijke klinische implicaties hebben. In de laatste jaren is meer en meer inspanning geleverd om behandelingen voor de ZvA te ontwikkelen. De heterogene natuur van de ziekte suggereert dat het niet erg waarschijnlijk is dat alle Alzheimerpatiënten baat zullen hebben bij eenzelfde behandeling. Aangezien "gepersonaliseerde geneeskunde" een steeds grotere rol gaat spelen, lijkt het steeds belangrijker om betekenisvolle subtypes te kunnen identificeren, welke allen een verschillende benadering nodig zullen hebben. Onze resultaten suggereren dat variabiliteit in klinische manifestatie binnen de ZvA samenhangt met biologische substraten, welke mogelijk wijzen op verschillende banen waardoor de ziekte tot uiting komt. Het is denkbaar dat het combineren van cognitieve profielen en biologische markers behulpzaam kan zijn in de toewijzing-op-maat van toekomstige behandelingen.

Veel geneesmiddelentrials gebruiken cognitieve verandering als primaire uitkomstmaat wanneer ze de effectiviteit van het betreffende geneesmiddel onderzoeken. Dit proefschrift heeft aangetoond dat de aanvangsleeftijd, het APOE genotype en CSF biomarkers allen samenhangen met de snelheid van cognitieve achteruitgang in de ZvA. Het is daarom aan te raden om rekening te houden met deze factoren wanneer nieuwe interventies geëvalueerd worden. In contrast hiermee is bijvoorbeeld de interesse in de klinische consequenties van microbloedingen onlangs toegenomen sinds amyloid gerelateerde beeldvormingabnormaliteiten (ARIA), waaronder de afzetting van hemosiderine (ARIA-H), zijn opgetreden bij patiënten die deelnamen aan klinische trials met agentia gericht op het verminderen van de hoeveelheid amyloid-ß in Alzheimerpatiënten.[19] In dit kader is onze vondst van een gebrek aan verband tussen microbloedingen (als uitingsvorm van ARIA-H) en de snelheid van cognitieve achteruitgang mogelijk van belang. Als de snelheid van cognitieve achteruitgang niet wordt beïnvloed door de aanwezigheid van of het aantal microbloedingen, is het waarschijnlijk niet nodig om patiënten met microbloedingen uit te sluiten van dergelijke trials. Het moet echter wel opgemerkt worden dat de prognose van ARIA-H wellicht verschilt van spontaan optredende microbloedingen. Het is daarom nodig om verder onderzoek te doen naar het risico op versnelde cognitieve achteruitgang in patiënten met ARIA-H.

Richtingen voor toekomstig onderzoek

In de literatuur zijn verschillende pogingen gedaan om subtypes in verschillende modaliteiten (bijv. cognitie, MRI, CSF) te definiëren.[20-25] In dit proefschrift hebben we patiënten ingedeeld op basis van biologische en pathofysiologische factoren en hebben we onderzocht of dit resulteerde in verschillende neuropsychologische profielen. De volgende stap is om de data van de andere kant te benaderen, door eerst vast te stellen in welke cognitieve clusters Alzheimerpatiënten ingedeeld kunnen worden. Het clusteren van cognitieve data zal dan moeten plaatsvinden op een op de data gebaseerde manier ('data driven'), zonder a priori hypothesen over het aantal of de aard van de subpopulaties, en de resultaten zullen moeten worden gevalideerd in een andere steekproef om hun overdraagbaarheid te testen. Vervolgens

zou in een meer holistische benadering gepoogd moeten worden om de gevonden subtypes te relateren aan een combinatie van biologische en pathofysiologische factoren zoals APOE genotype, CSF biomarkers, EEG afwijkingen, MRI maten en aanvangsleeftijd.

Bovendien zal er - naast het bestuderen van verbanden tussen cognitieve fenotypen en bekende biologische en pathofysiologische factoren - veel energie gestoken moeten worden in het vinden van nieuwe biomarkers in bloed, hersenvocht of andere lichaamssappen. We hebben biomarkers nodig die de voortgang van de ziekte (prognostische markers) en de respons op behandeling (theragnostische markers) kunnen voorspellen. Biologische factoren die betekenisvolle variatie vertonen binnen de ZvA zijn hier waarschijnlijke goede kandidaten voor. Het is bijvoorbeeld een algemeen geaccepteerd idee dat andere genetische factoren naast het APOE genotype de pathogenese van de sporadische vorm van de ZvA beïnvloeden. Tot op heden hebben genoomwijde associatiestudies zich met name gericht op het identificeren van factoren die betrekking hebben op de gevoeligheid voor de ziekte.[26] De volgende stap is om - in plaats van het vergelijken van het DNA van Alzheimerpatiënten met dat van controle proefpersonen - het DNA van verschillende cognitieve subtypen van de ZvA met elkaar te vergelijken in een poging om genetische factoren te identificeren die de uiting van de ziekte beïnvloeden.

De lijn van de integratie van data over cognitieve subtypes met biologische, genetische en pathofysiologische factoren in een holistische benadering kan worden doorgetrokken naar de snelheid van cognitieve achteruitgang. In navolging op de studies uit dit proefschrift waarin gekeken is naar de relatie tussen individuele factoren zoals APOE genotype, CSF biomarkers en de aanvangsleeftijd, zou het interessant zijn om een algoritme te ontwikkelen dat gebaseerd is op deze en andere biologische, genetische en pathofysiologische factoren om zo de snelheid van cognitieve achteruitgang voor individuele Alzheimerpatiënten te kunnen voorspellen. Recentelijk zijn zogenaamde machine leeralgoritmen voorgesteld om verschillende Alzheimerkenmerken afkomstig uit beeldvormend en biomarkeronderzoek te integreren om zo onderscheid te kunnen maken tussen Mild Cognitive Impairment (MCI) en de ZvA en voor het voorspellen van de overgang van MCI naar de ZvA.[27] Verschillende studies hebben diagnostische classificatie op basis van verschillende MRI-maten mogelijk gemaakt.[28;29] Anderen hebben hierop voortgeborduurd door data van andere modaliteiten, zoals demografische variabelen als geslacht en leeftijd, maar ook APOE genotype, CSF biomarkers[30] en PET-data,[27;31;32] op te nemen. De volgende stap zou zijn om data uit verschillende modaliteiten te combineren om zo de snelheid van cognitieve achteruitgang te voorspellen als de patiënt zich eenmaal in het dementiestadium bevindt. Naast de evidente baten die het voor de patiënt en zijn/haar mantelzorgers oplevert wanneer we de snelheid van cognitieve achteruitgang kunnen voorspellen, zal dit ook vitale informatie opleveren voor het ontwerp, de powerberekening en de implementatie van toekomstige klinische trials.

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5

Remember me, but ah! forget my Fate.

Dido, aria *"When I am laid in Earth"*
Dido and Aeneas
Henry Purcell

6

CODA

List of abbreviations
Dankwoord
Over de auteur
List of publications
Hall of fame

| LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
ANOVA	Analysis of Variance
APOE	Apolipoprotein E
ARIA	Amyloid related imaging abnormalities
Aβ	Amyloid beta
CDR	Clinical Dementia Rating
CSF	Cerebrospinal fluid
GCA	Global cortical atrophy
LP	Lumbar puncture
MANOVA	Multivariate analysis of variance
MBs	Microbleeds
MCI	Mild Cognitive Impairment
MIS+	Memory Impairment Screen+
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging
MTA	Medial temporal lobe atrophy
TMT	Trail Making Test
VAT	Visual Association Test
WMH	White matter hyperintensities

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Coda

| DANKWOORD

Het werken bij het VUmc Alzheimercentrum is vanaf dag 1 een waar genoegen geweest! De positieve atmosfeer, het enthousiasme om goede zorg te leveren en een substantiële bijdrage te leveren aan het wereldwijde onderzoek naar dementie en niet te vergeten de mensen die samen het centrum vormen, zijn de componenten die dit tot zo'n mooie werkplek maken. Ik ben dan ook heel blij dat ik hier voorlopig nog rond mag lopen! Graag wil ik iedereen bedanken die op welke manier dan ook een bijdrage aan dit proefschrift heeft geleverd.

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het zingen te gaan besteden, verzuchtte je dat je gedacht had dat we samen 65 zouden worden. En nu, wie weet, "When I'm sixty-four"? Misschien komt het er wel van…

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Argonde	Floor	Marije	Sietske
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SDG

| OVER DE AUTEUR

Annelies Vonk Noordegraaf - van der Vlies werd op 20 december 1977 geboren in Kapelle (Zld). In 1996 ontving ze haar VWO diploma aan het Buys Ballot College te Goes. Na een kleine omzwerving via de studie Creatieve Therapie aan de Hogeschool van Utrecht begon ze in 1998 aan haar studie Psychologie aan de Faculteit Sociale Wetenschappen van de Universiteit Utrecht, met als afstudeerrichting Neuropsychologie. Bij de afdeling Neurologie/Neuropsychologie van het LUMC verrichtte zij zowel haar klinische als haar wetenschappelijke stage.

Na het behalen van haar doctoraalbul werkte Annelies als neuropsychologisch test assistent bij de afdeling Neurologie van het UMC Utrecht en als onderwijsassistent bij de vakgroep Psychonomie van de Universiteit Utrecht. In 2004 begon zij een studie Klassieke Zang aan het Conservatorium van Amsterdam, alwaar zij in 2009 de graad Bachelor of Music verkreeg. Sinds 2005 is zij werkzaam bij het VUmc Alzheimercentrum. Vanaf het begin van haar aanstelling is zij betrokken bij de opzet en het management van de database van het Alzheimercentrum. Deze database, met daarin gegevens van ruim 6.000 patiënten, speelt een cruciale rol binnen het onderzoek dat binnen het VUmc Alzheimercentrum verricht wordt. In het eerste jaar van haar aanstelling was Annelies tevens als neuropsycholoog betrokken bij het diagnostisch patiënten onderzoek binnen het VUmc Alzheimercentrum. In 2006 is zij begonnen met haar eigen onderzoek, wat geleid heeft tot dit proefschrift. Momenteel houdt Annelies zich, naast het databasemanagement, bezig met de kwaliteit en integriteit van het wetenschappelijk onderzoek dat binnen het VUmc Alzheimercentrum verricht wordt.

Annelies is getrouwd met Ton Vonk Noordegraaf en in 2012 kregen zij samen een dochter, Benthe.

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Coda

| LIST OF PUBLICATIONS

International publications

Koedam ELGE, **van der Vlies AE**, van der Flier WM, Verwey NA, Koene T, Scheltens P, Blankenstein MA, Pijnenburg YAL. Cognitive correlates of cerebrospinal fluid biomarkers in frontotemporal dementia. *Alzheimers Dement.* 2013 May;9(3):269-75.

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| HALL OF FAME

VUmc Alzheimer Center PhD theses

L. Gootjes: Hemispheric connectivity and laterality of language processing (14-9-2004)

R. Goekoop: Pharmacological fMRI: a clinical exploration (16-01-2005) (Cum Laude)

K. van Dijk: Peripheral electrical nerve stimulation in Alzheimer's Disease (6-9-2005)

N.S.M. Schoonenboom: Cerebrospinal fluid markers for the early and differential diagnosis of Alzheimer's disease (10-11-2006)

E.S.C. Korf: Medial Temporal Lobe atrophy on MRI: vascular risk factors and predictive value in dementia (29-11-2006)

B. van Harten: Aspects of subcortical vascular ischemic disease (22-12-2006).

B. F. Jones: Cingular cortex networks (23-03-2007)

L. van de Pol: Hippocampal atrophy from aging to dementia: a clinical perspective (11-05-2007)

Y.A.L. Pijnenburg: Frontotemporal dementia: towards an early diagnosis (5-7-2007)

A. J. Bastos Leite: Pathological ageing of the Brain (16-11-2007)

E.C.W. van Straaten: MRI correlates of vascular cerebral lesions and cognitive impairment (11-1-2008)

R.L.C. Vogels: Cognitive impairment in heart failure (11-4-2008)

J. Damoiseaux: The brain at rest (20-5-2008)

G.B. Karas: MRI patterns of cerebral atrophy in dementia (19-6-2008)

F.H. Bouwman: Biomarkers in dementia: longitudinal aspects and combination with MRI (20-6-2008)

A.A. Gouw: Cerebral small vessel disease on MRI (20-03-2009)

H. van der Roest: Care needs in dementia and interactive digital information provisioning (12-10-2009)

C. Mulder: Biomarkers in Alzheimer's disease (11-11-2009)

W. Henneman. Advances in hippocampal atrophy measurement in dementia: beyond diagnostics (27-11-2009)

S.S. Staekenborg: Risk factors and clinical findings in relation to vascular changes on brain MRI (23-12-2009)

N. Tolboom: Imaging Alzheimer's disease pathology in vivo: towards an early diagnosis (12-02-2010)

E. Altena: Mapping insomnia: brain structure, function and sleep intervention (17-03-10).

N.A. Verwey: Biochemical markers in dementia: from mice to men (15-04-2010)

M.I. Kester: Biomarkers for Alzheimer's pathology; monitoring, predicting and understanding the disease (14-01-2011)

J.D. Sluimer: Visualizing the shrinking brain (28-04-11).

S.D Mulder: Amyloid associated proteins in Alzheimer's disease (07-10-11)

S.A.M. Sikkes:	Measuring IADL in dementia (14-10-11)
A. Schuitemaker:	Inflamation in Alzheimer's Disease: in vivo quantification (27-01-12)
K. Joling:	Depression and anxiety in family caregivers of persons with dementia (2-4-12)
W. De Haan:	In a network state of mind (02-11-12) (Cum Laude)
D. Van Assema:	Blood-brain barrier P-glycoprotein function in aging and Alzheimer's disease (07-12-12)
J. D.C. Goos:	Cerebral microbleeds: connecting the dots (6-2-13)
R. Ossenkoppele:	Alzheimer PEThology (8-5-13)
H.M. Jochemsen:	Brain under pressure: influences of blood pressure and angiotensin-converting enzyme on the brain (4-10-13)

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