frontiers

# The assessment of visuospatial skills and verbal fluency in the diagnosis of Alzheimer's disease

Dalida B. Berente[1], Anita Kamondi[1], Andras A. Horvath[2*]

[1]Semmelweis University, Hungary, [2]National Institute of Mental Health, Neurology and Neurosurgery, Hungary

*Submitted to Journal:*
Frontiers in Aging Neuroscience

*Article type:*
Original Research Article

*Manuscript ID:*
737104

*Received on:*
06 Jul 2021

*Journal website link:*
www.frontiersin.org

## Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

## Author contribution statement

B.D.B.was responsible for data management and the conduction of statistical analysis. She contributed to the writing of the manuscript.
A.K.was involved in the recruitment of patients, and in the design of the study protocol. She contributed to the correction of the manuscript.
A.A.H. performed neuropsychological assessments, evaluated the results and concluded the major findings. He contributed to the writing of the manuscript.


## Keywords

Alzheimer' s disease, Neuropsychology, verbal fluency, Visuospatial abilities, Cognitive domain




## Abstract


Word count: 261
Background: In the diagnosis of Alzheimer's disease (AD), examining memory is predominant. Our aim was to analyse the potential role of various cognitive domains in the cognitive evaluation of AD.
Methods: 110 individuals with clinically defined AD and 45 healthy control participants underwent neuropsychological evaluation including Addenbrooke's Cognitive Examination (ACE). AD patients were selected in three groups based on disease duration in years (y) (Group 1: ≤2y n=36; Group 2: 2-4y n=44, Group 3: ≥4y n=30). Covariance weighted intergroup comparison was performed on global cognitive score and subscores of cognitive domains. Spearman's rho was applied to study the correlation between cognitive subscores and disease duration. Wilcoxon signed ranked test was used for within group analysis among ACE cognitive subscores.
Results: Significant difference was found between ACE total scores among groups (x2=119,1; p<0,001) with a high negative correlation (p<0,001; r: -0,643). With longer disease duration, all the subscores of ACE significantly decreased (p's <0,001). Visuospatial score showed the strongest negative correlation with disease duration with a linear trajectory in decline (r: -0,85). In the early phase of cognitive decline, verbal fluency was the most impaired cognitive subdomain (normalized value: 0.64), and it was significantly reduced compared to all other subdomains (p's<0.05).
Conclusion: We found that impairment of verbal fluency is the most characteristic feature of early cognitive decline, therefore it might have crucial importance in the early detection of Alzheimer's disease. Based on our results visuospatial assessment might be an ideal marker to monitor the progression of cognitive decline in AD.
Key words: (3-5): Alzheimer's disease, neuropsychology, cognitive domains, progression, diagnosis
Introduction


## Contribution to the field

While the role of memory impairment is a frequently observed aim of research studies, lower number of studies have investigated the importance of visuospatial abilities and verbal fluency in the early recognition of Alzheimer's disease and in the monitoring of progression of cognitive decline. In the current study, we analyzed the cognitive profile of 110 rigorously selected Alzheimer patients with various disease duration and 45 healthy controls. We analyzed the contribution of six cognitive domains in the cognitive deficit of Alzheimer patients, namely orientation, memory, language, attention, verbal fluency and visuospatial abilities. We demonstrated that verbal fluency is the most impaired cognitive subdomain in the initial phases of AD. We also highlighted that only visuospatial scores follow a linear decline among the disease course indicating the priority of this cognitive domain in assessment of disease progression.


## Funding statement

Our study was supported by National Brain Research Program I, II (KTIA_NAP_13-1-2013-0001; 2017-1.2.1-NKP-2017-00002), Hungarian Scientific Research Fund 2019 of the National Research, Development and Innovation Office (PD- 132652), Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences (bo_78_20_2020), New National Excellence Program of the National Research, Development and Innovation Office (ÚNKP-20-5-SE-16). This is an EU Joint Programme- Neurodegenerative Disease Research (JPND) project. The project is supported through the following funding organization under the aegis of JPND-

www.jpnd.eu (National Research, Development and Innovation Office, Hungary, 2019-2.1.7-ERA-NET-2020-00006).

## *Ethics statements*

### *Studies involving animal subjects*

Generated Statement: No animal studies are presented in this manuscript.

### *Studies involving human subjects*

Generated Statement: The studies involving human participants were reviewed and approved by The Hungarian Medical Research Council . The patients/participants provided their written informed consent to participate in this study.

### *Inclusion of identifiable human data*

Generated Statement: No potentially identifiable human images or data is presented in this study.

## *Data availability statement*

Generated Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

# The assessment of visuospatial skills and verbal fluency in the diagnosis of Alzheimer's disease

**Dalida Borbala Berente[1,2], Anita Kamondi MD, PhD[2,3], Andras Attila Horvath MD, PhD[2,4*]**

[1]Semmelweis University Faculty of Medicine, Budapest, Hungary

[2]National Institute of Mental Health, Neurology and Neurosurgery, Neurocognitive Research Center, Budapest, Hungary

[3]Semmelweis University Department of Neurology, Budapest, Hungary

[4]Semmelweis University, Department of Anatomy Histology and Embryology, Budapest, Hungary

*** Correspondence:**
Andras Attila Horvath, MD, PhD
57 Amerikai út
1145-Budapest, Hungary
Phone: +36305421019
horvath.andras1@med.semmelweis-univ.hu

Main text word count: 3880

Number of figures: 3

Number of tables: 2

**Key words: Alzheimer's disease, neuropsychology, cognitive domains, progression, diagnosis**

## Abstract

**Background:** In the diagnosis of Alzheimer's disease (AD), examining memory is predominant. Our aim was to analyse the potential role of various cognitive domains in the cognitive evaluation of AD.

**Methods:** 110 individuals with clinically defined AD and 45 healthy control participants underwent neuropsychological evaluation including Addenbrooke's Cognitive Examination (ACE). AD patients were selected in three groups based on disease duration in years (y) (Group 1: ≤2y n=36; Group 2: 2-4y n=44, Group 3: ≥4y n=30). Covariance weighted intergroup comparison was performed on global cognitive score and subscores of cognitive domains. Spearman's rho was applied to study the correlation between cognitive subscores and disease duration. Wilcoxon signed ranked test was used for within group analysis among ACE cognitive subscores.

**Results:** Significant difference was found between ACE total scores among groups ($\chi 2$=119,1; p<0,001) with a high negative correlation (p<0,001; r: -0,643). With longer disease duration, all the subscores of ACE significantly decreased (p's <0,001). Visuospatial score showed the strongest negative correlation with disease duration with a linear trajectory in decline (r: -0,85). In the early phase of cognitive decline, verbal fluency was the most impaired cognitive subdomain (normalized value: 0.64), and it was significantly reduced compared to all other subdomains (p's<0.05).

**Conclusion**: We found that impairment of verbal fluency is the most characteristic feature of early cognitive decline, therefore it might have crucial importance in the early detection of Alzheimer's disease. Based on our results visuospatial assessment might be an ideal marker to monitor the progression of cognitive decline in AD.

Key words: (3-5): Alzheimer's disease, neuropsychology, cognitive domains, progression, diagnosis



## Introduction

Currently there are around fifty million patients worldwide living with major neurocognitive disorders. This number is expected to triple by 2050, placing tremendous socio-economic and medical burden on the society. Alzheimer's disease (AD) is the leading cause of cognitive decline in older adults, accounting for two thirds of dementia cases worldwide (1). AD is characterised by gradual decline of cognitive function, affecting the social and communication skills as well. The histopathological hallmarks of the disease are the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles (2). The initially affected neural structures are the hippocampus and the entorhinal cortex (3). These areas have crucial role in episodic memory, spatial orientation, and visuospatial abilities.

The progression of the disease follows a pattern starting with mild cognitive impairment (MCI) as the prodromal phase of AD which may appear years prior to the dementia diagnosis of a patient. In most patients, MCI is characterised by memory complaints (amnestic type MCI) (4). According to the current DSM-V diagnostic guideline, short-term memory impairment becomes significant and learning difficulties appear in mild AD (5). In moderate AD, other cognitive domains are involved as well including language difficulties and impaired orientation. In severe AD, all cognitive domains are severely affected, communication skills and self-reliance are lost (6).

Current diagnostic guidelines advise the evaluation of a patient's medical history, clinical examination to test mental status as core tests and cerebrospinal fluid analysis, neuroimaging

using magnetic resonance imaging or positron emission tomography as supportive diagnostic markers (7). Use of neuropsychological test batteries is recommended too (e.g. Montreal Cognitive Assessment- MoCA, Addenbrooke Cognitive Examination- ACE, Alzheimer's Disease Assessment Scale-Cognitive Subscale- ADAS-Cog). These tests focus mostly on assessing memory function and learning skills (the ratio of memory points/ maximum score is 5/30 in MoCA, 35/100 in ACE and 35/70 in ADAS-Cog), while investigation of visuospatial abilities (the ratio of visuospatial points/ maximum score is 4/30 in MoCA, 5/100 in ACE and 0/70 in ADAS-Cog), and verbal fluency (the ratio of verbal fluency points/ maximum score is 1/30 in MoCA, 14/100 in ACE and 5/70 in ADAS-Cog), is relatively less detailed (8). However, they might hold significant diagnostic and prognostic potential as well (9) since they require organized activation of large neural networks (10-12).

We hypothesised that in AD the severity of visuospatial- and verbal fluency performance decline is related to disease duration, as during the course of the neurodegenerative process more and more cortical areas involved in these functions become affected. Thus, our aim was to analyse the profile of cognitive impairment in AD patients with various disease duration exploring multiple cognitive domains (memory, orientation, attention, verbal fluency, language and visuospatial abilities) to assess their potential role in the early identification of AD and in the follow-up of the progression of cognitive decline.

## Methods

### Participants

One hundred and ten participants (61 male, 49 female, mean age 73,1±6,6) with clinically defined AD and fourty-five healthy control participants (16 male, 29 female, mean age 68,6 ±7,40) were recruited from the Department of Neurology at the National Institute of Mental Health, Neurology and Neurosurgery (previously named National Institute of Clinical Neurosciences) in Budapest, Hungary. Informed written consent was obtained from each participant. The participants' diagnosis was given based on the guidelines of the National Institute on Aging and the Alzheimer's Association (NIA-AA). (13) We sorted the participants with AD in three groups based on disease duration. Group 1 (n=36) included participants with disease duration up to two years, group 2 (n=44) with disease duration of 2 to 4 years, and group 3 (n=30) with disease duration of 4 years or more. The healthy control individuals (Group 0; n=45) had negative neurological status and intact cognitive performance based on neuropsychology. Disease duration was calculated from the date of clinical diagnosis of AD. Heteroanamnestic data were also collected from family members and caregivers. Patients with a history of cognitive symptoms more than 2 years prior to the diagnosis of AD were not included in the current analysis. All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by The Hungarian Medical Research Council (reference number of ethical approval: 024505/2015).

### Clinical testing

The participants underwent detailed medical, neurological, physical examination, as well as routine blood checks including thyroid functions and vitamin B12 levels. All patients had structural brain magnetic resonance imaging (MRI). MRIs were analyzed with visual inspection and medial temporal lobe atrophy (MTA) score was calculated. MTA=1 shows that choroid fissure is slightly widened among the hippocampi, MTA=2 shows mild enlargement of temporal horn and mild loss of hippocampal height, MTA=3 indicates moderate enlargement of temporal horn and moderate loss of hippocampal height, while MTA=4 shows the marked enlargement of temporal horn and the loss of internal hippocampal structure. (14) We

determined all the known risk factors of cognitive decline as exclusion criteria. Such risk factors included: untreated vitamin B12 deficiency or hypothyroidism, liver disease, renal insufficiency, alcohol or substance abuse, psychoactive drugs influencing cognitive function except for anti-dementia medications, clinically significant brain lesions (white matter lesions, stroke,) demyelinating conditions, head injury with loss of consciousness, hydrocephalus, schizophrenia, major depression, electroconvulsive therapy, HIV infection, syphilis or prior central nervous system infections.

Neuropsychology

All participants took part in neuropsychological evaluation. The assessments were conducted by trained neurologists or neuropsychologists. The language of evaluation was Hungarian. We selected the Hungarian version of Addenbrooke's Cognitive Examination (ACE) (15) to assess cognitive function. It is known for its high specificity and sensitivity in the diagnosis of cognitive disorders (16). It tests six cognitive domains: orientation, attention, memory, verbal fluency, language and visuospatial abilities with a maximum score of 10; 8; 35; 14; 28; 5 respectively, resulting in a maximum total score of 100. A total score of 83 set as cut off score has a 82% sensitivity at age>65 (17). Calculating the ratio of verbal fluency (V) and language (L) subscores/orientation (O) and delayed recall memory (M) subscores (VLOM ratio: (V+L)/(O+M)) enables differentiation between AD and frontotemporal dementia (FTD). The normal range of VLOM ratio is between 2.2 and 3.2. A value higher than 3.2 indicates Alzheimer-type dementia, while a value lower than 2.2 demonstrates frontotemporal type dementia. Visuospatial abilities are tested by asking the participant to copy two overlapping pentagons, to copy a cube and to draw a clock face with the hands set at a specified time. Verbal fluency is analyzed with two tasks to examine categorical fluency (naming of animals) and phonemic fluency (listing words starting with the letter "m"). Furthermore, the Mini-Mental State Examination (MMSE) is incorporated in the ACE enabling dementia severity assessment. Its total score ranges from 0 to 30, with higher scores indicating better cognitive performance. AD patients had MMSE<25, while controls had>25.

Depression and anxiety may impair cognitive function (18, 19). To reduce the influence of depression and axiety on the data, we included the Beck Depression Inventory II (BDI-II) and Spielberger State and Trait Anxiety Inventory (STAI) in our test battery. A BDI-II score of less than 13 demonstrates minimal depression. Scores between 14 and 19 indicate mild depression, those between 20 and 28 refer to moderate depression, while a score of 29 or higher demonstrates severe depression. A low level of anxiety is indicated by a score of 45 or less for both state and trait anxiety. Participants with a BDI-II score of >13 or a STAI score of >45 were excluded from our analysis.

Data analysis

A recent study of de Boer et al. (20) reported significant differences in MMSE total score and cognitive subdomains' scores between three study groups of 125 AD patients in total with various disease durations. Based on their results and our power calculations the probability was equal or greater than 80% to find a significant (alpha=0.05) difference between study groups in ACE total and cognitive subscores with a sample size of 150. Data distribution was tested using Shapiro-Wilk test. To test for significant differences (for intergroup comparisons) in demographic variables (e.g. age, years of education) one-way ANOVA and Kruskal-Wallis tests were used as parametric and non-parametric tests respectively based on the distribution of data. Statistical significance level was set at $p<0.01$ based on Bonferroni-correction due to

multiple comparisons. Due to the non-parametric distribution of data Spearman's rho was used to study the correlation between disease duration (years) and cognitive function represented by the ACE total score. Between-group differences for ACE subscores were tested with covariance weighted (age, sex, disease onset) ANOVA and Kruskal-Wallis tests. Tukey test was applied for post-hoc analysis. Spearman correlation was applied for the connection of ACE subscores and disease duration. For within group analysis including normalized ACE subscores, normalization was applied with the achieved score in each cognitive domain divided with a maximum possible score of the same cognitive domain (e.g., 7/28 in language cognitive domain resulted in 0.25). Normalized data were compared with Wilcoxon-signed rank test because of the non-parametric distribution. IBM SPSS 20 software was used for statistical analysis.

## Results

### Demographic data

Altogether 155 individuals (77 male: 49,7%, 78 females: 50,3%) participated in the study. The participants' mean age was 71,8±7,1 years. The median duration of their education was 12 (12,0-17,0) years. Of the 155 participants 45 were cognitively intact control individuals while 110 were diagnosed with clinically defined Alzheimer's disease. On brain MRI patients showed the characteristic cortical atrophy (bifrontal-bitemporal atrophy with reduced hippocampi). All patients had MTA score ≥ 3.

Group 1 (n=36; disease duration of no more than 2 years) included 23 male (63,89%) and 13 female (36,11%) participants with a mean age of 70,7±7,4 years. In group 2 (n=44; disease duration of 2 to 4 years) there were 25 male (56,8%) and 19 female (43,2%) participants. Their mean age was 74,1±6,2 years. In group 3 (n=30; disease duration longer than 4 years) 13 male (43,3%) and 17 female (56,7%) participants were selected, with a mean age of 74,6±5,4 years. Group 0 included 45 control individuals (16 male (35,6%) and 29 female (64,4%)). Their mean age was 68,6±7,4 years. We studied between-group differences in sex, age, age at disease onset, education level, disease duration, ACE total score, ACE subscores and VLOM ratio (Table 1). Significant differences ($p<0.001$) were reported in almost all parameters except sex and age at disease onset.

Insert Table 1.

### Relationship between ACE total score and disease duration

Spearman's rho showed a significant negative correlation between ACE total scores and disease duration ($p<0,001$; r:-0,643). To support this finding a one-way Kruskal-Wallis test was used confirming significant group effect on total ACE score ($\chi2=115,81$; $p<0,001$).

### Between-group differences between ACE subscores

One-way ANOVA was used to test between-group differences between the memory subscores. (Table 1). Significant between-group differences were found for memory ($F=69,11$; $p<0,001$). Kruskal-Wallis test was applied to study between-group differences between subscores of orientation, attention, verbal fluency, language, and visuospatial abilities (Table 1). Significant between-group differences were found for orientation ($\chi2= 96,27$; $p<0,001$), attention ($\chi2= 87,11$; $p<0,001$), verbal fluency ($\chi2= 61,12$; $p<0,001$), language ($\chi2=100,38$; $p<0,001$) and visuospatial abilities ($\chi2=113,96$; $p<0,001$). Age, sex and disease onset did not have significant modifier effect on between group differences (all p values>0.01). Tukey post-hoc analysis revealed that Group 1 differs from Group 2, Group 3 and Group 0 in orientation

skills (all p values<0.001). Group 0 also differs from Group 2 and Group 3 in orientation skills (all p values <0,001) however, Group 2 and Group 3 are not significantly different (p=0,779). In terms of attention subscore, Group 1, Group 3 and Group 0 all differ from each other significantly (all p values <0,001). Group 2 differs from Group 3 and Group 0 significantly (all p values <0,001). However, Group 1 and Group 2 do not differ significantly (p=0,984). As for memory subscore, Group 2, Group 3 and Group 0 all differ from each other significantly (all p values <0,001). Group 1 differs from Group 3 and Group 0 significantly (all p values <0,001). However, Group 1 and Group 2 do not differ significantly (p=0,254). Regarding the subscore of verbal fluency, Group 0 differs from Group 1, Group 2 and Group 3 (all p values <0,001). However, Group 1 does not differ significantly from Group 2 and Group 3 (p=0,629 and p=0,017 respectively). Moreover, Group 2 does not differ significantly from Group 3 (p=0,198). Concerning language subscore, Group 1, Group 3 and Group 0 all differ from each other significantly (all p values <0,001). Group 2 differs from Group 1 and Group 0 significantly (all p values <0,001). However, Group 2 and Group 3 do not differ significantly (p=0,142). In terms of visuospatial subscore, all four groups differed significantly (all p values ≤ 0,001). (Figure 1). In the comparison to normal controls (Group 0), verbal fluency showed the largest difference in the first phase of the disease (Group 1).

Insert Figure 1.

Relationship between ACE subscores and disease duration

Spearman's rho was applied to test the relationship between all six ACE subscores and disease duration. Figure 2 demonstrates scatter plots for subscores in relation to disease duration (Figure 2).

Insert Figure 2.

Within-group differences between ACE subscores

We applied Wilcoxon signed-ranked test for within-group difference analysis between ACE subscores. Differences between the normalized subscores are shown in Figure 3 and Table 2.

In Group 0 normalized subscore of orientation was significantly higher than the normalized subscore of memory (Z: -4,083; p<0,001), verbal fluency (Z:-3,95; p<0,001) and visuospatial abilities (Z: -2,10; p=0,036). However, the normalized subscore of orientation was significantly lower than the normalized subscore of language (Z: -2,32; p=0,021). There was no significant difference between the normalized subscores of orientation and attention. Normalized subscore of attention is significantly higher than the normalized subscore of memory (Z: -5,40; p<0,001), verbal fluency (Z: -4,60; p<0,001) and visuospatial abilities (Z: -2,94; p=0,003). There was no significant difference between the normalized subscores of attention and language. Normalized subscore of memory was significantly lower than the normalized subscore of language (Z: -5,52; p<0,001) and visuospatial abilities (Z: -3,61; p<0,001). There was no significant difference between the normalized subscores of memory and verbal fluency. Normalized subscore of verbal fluency was significantly lower than the normalized score of language (Z: -4,68; p<0,001) and visuospatial abilities (Z: -3,75; p<0,001). Normalized subscore of language was significantly higher than the normalized subscore visuospatial abilities (Z: -2,82; p=0,005).

In Group 1 normalized subscore of orientation was significantly higher than the normalized subscore of attention (Z: -2,34; p=0,019), memory (Z: -2,27; p=0,023) and verbal fluency (Z: -4,79; p<0,001). There was no significant difference between the normalized

subscores of orientation, language and visuospatial abilities. Normalized subscore of attention was significantly higher that the normalized subscore of verbal fluency (Z: -4,14; p<0,001). However, normalized subscore of attention was significantly lower than the normalized subscore of language (Z: -5,23; p<0,001). There was no significant difference between the normalized subscores of attention, memory and visuospatial abilities. Normalized subscore of memory was significantly higher than the normalizes subscore of verbal fluency (Z: -4,41; p<0,001). However, normalized subscore of memory was significantly lower than the normalized subscore of and language (Z: -5,23; p<0,001). There was no significant difference between the normalized subscores of memory and visuospatial abilities. Normalized subscore of verbal fluency was significantly lower than the normalized subscore of language (Z: -5,23; p<0,001) and visuospatial abilities (Z: -4,69; p<0,001). There was no significant difference between the normalized subscores of language and visuospatial abilities.

In Group 2 normalized subscore of orientation was significantly higher than the normalized subscore of verbal fluency (Z: -3,62; p<0,001) and visuospatial abilities (Z: -4,38; p<0,001). However, normalized subscore of orientation was significantly lower than the normalized subscore of attention (Z: -3,23; p=0,001), memory (Z: -2,19; p=0,029). There was no significant difference between the normalized subscores of orientation and language. Normalized subscore of attention was significantly higher that the normalized subscore of verbal fluency (Z: -5,47; p<0,001), language (Z: -2,14; p=0,032) and visuospatial abilities (Z: -5,24; p<0,001). There was no significant difference between the normalized subscores of attention and memory. Normalized subscore of memory was significantly higher than the normalized subscore of verbal fluency (Z: -4,87; p<0,001), and visuospatial abilities (Z: -5,25; p<0,001). There was no significant difference between the normalized subscores of memory and language. Normalized subscore of verbal fluency was significantly higher than the normalized subscore of visuospatial abilities (Z: -3,31; p=0,001). However, normalized subscore of verbal fluency was significantly lower than the normalized subscore of language (Z: -3,55; p<0,001). Normalized subscore of language was significantly higher than the normalized subscore visuospatial abilities (Z: -4,07; p<0,001).

In Group 3 normalized subscore of orientation was significantly higher than the normalized subscore of memory (Z: -3,86; p<0,001), verbal fluency (Z: -3,75; p<0,001) and visuospatial abilities (Z: -4,73; p<0,001). There was no significant difference between the normalized subscores of orientation, attention and language. Normalized subscore of attention was significantly higher than the normalized subscore of memory (Z: -3,10; p=0,002), verbal fluency (Z: -2,42; p=0,016) and visuospatial abilities (Z: -4,74; p<0,001). There was no significant difference between the normalized subscores of attention and language. Normalized subscore of memory was significantly higher than the normalized subscore of visuospatial abilities (Z: -4,46; p<0,001). However, normalized subscore of memory was significantly lower than the normalized subscore of language (Z: -4,32; p<0,001). There was no significant difference between the normalized subscores of memory and verbal fluency. Normalized subscore of verbal fluency was significantly higher than the normalized subscore of visuospatial abilities (Z: -4,47; p<0,001). However, normalized subscore of verbal fluency was significantly lower than the normalized subscore of language (Z: -2,76; p=0,006). Normalized subscore of language was significantly higher than the normalized subscore visuospatial abilities (Z: -4,64; p<0,001).

Insert Table 2 and Figure 3.

## Discussion

Our study involved 110 clinically defined AD patients divided into three groups based on the length of disease duration. The control group (Group 0) consisted of 45 cognitively intact individuals. We found that verbal fluency is the most impaired cognitive domain in the first 2 years of the disease course, and its disturbance is comparable to the memory impairment in the early phase of AD. Furthermore, since visuo-spatial abilities showed the most linear reduction among the groups with various disease lengths, it might serve as an ideal method for monitoring disease progression.

Our analysis using correlation and between-group approaches showed that patients with longer disease duration have lower ACE global scores being in line with the current literature and confirming the fact that ACE indicates well the severity of AD (21) and global decline in cognition most frequently shows a linear pattern in AD (22, 23).

While significant reduction in ACE subscores were present in a more advanced disease stage in case of memory, verbal fluency, language, orientation, attention, and visuospatial abilities; the pattern of the impairment of various cognitive domains demonstrated prominent differences. Other studies also showed that selective analysis of cognitive subdomains might reveal various trajectories of cognitive decline in AD (23). Episodic memory impairment is the hallmark of AD; however, controversial results exist. Some reports suggest that declined episodic memory functions associate with the early phase of AD (24, 25) while others suggest that prominent impairment occurs in the advanced phase of cognitive decline (6, 20). Our findings might reveal a deeper insight to the proposed problem. Our results show that memory is a highly affected cognitive domain already in the early course of the disease having significantly lower normalized score (0.78) than any other subscores except attention (0.77) and verbal fluency (0.64). However, during the first 2-3 years after the diagnosis the subsequent decrease of memory scores is not prominent (Group 1 and 2 do not differ significantly in these subscores) suggesting that sequential memory testing might not be the ideal tool to sensitively detect the progression of the cognitive decline. However, memory functions show rapid decline after 4 years of disease onset supporting earlier data that demonstrated that memory impairment is predominantly evident in the later stages of AD (20). This might suggest that while the global cognitive decline shows a continuously progressive course with the duration of the disease, episodic memory loss is becoming less pronounced while other domains contribute more in the linear global decline. From these data we might conclude that testing memory independently is not appropriate to monitor disease progression or estimate the effect of disease modifying interventions and drug trials in the mild and moderate phases of AD.

We also found that verbal fluency was even more severely compromised at the early stage of AD than memory (0.78 normalized score for memory vs 0.64 normalized score for verbal fluency). Other reports also highlighted that verbal fluency is impaired even in amnestic type MCI (26), in the preclinical phase or mild phase of AD (27). Ideal verbal fluency tests could not be developed for routine screening of cognitive decline since there are controversial results: some studies propose that semantic (category) fluency might be an ideal tool for the early screening of dementia (28-30) while others demonstrated the superiority of phonemic (letter) fluency (26). However, a meta-analysis of 153 studies with 15990 participants proposed that semantic deficit is more prominent than phonemic (31). Based on our observations, it seems feasible that development of novel and more focused diagnostic procedures on verbal fluency might be an important direction for the early screening of cognitive decline.

Our correlation analysis between disease duration and ACE subscores showed that patients with longer disease duration perform worse in all cognitive subdomain test. Visuospatial score showed remarkably strong negative correlation (larger than any other domains) with disease duration (r:-0.85) drawing special attention to this cognitive domain. Visuospatial skills are used to remember directions, addresses, and layout of familiar places. Visuospatial abilities are tested by asking the patient to copy two diagrams; to draw a clock

face with the hands set at a specified time; to count sets of dots; and to recognize four letters which are partially obscured. Although problems in visuospatial abilities are less well characterised symptoms of AD compared to memory impairment (9), visuospatial function monitoring could be ideal for assessing whether cognitive decline is progressive or not. Furthermore, it might be a useful cognitive test for outcome measures of drug trials or lifestyle interventional studies.

There are limitations to our study. Firstly, positron emission tomography, cerebrospinal fluid analysis or genetic testing were not applied in the current experiment. Furthermore, cognitive decline might appear years preceding the diagnosis of AD, so disease duration might vary among the examined patients. We involved patients with short history of cognitive decline prior to the diagnosis of AD based on the reports of caregivers, however opinion of family members could be subjective. The strength of our study is the rigorous patient selection and the extensive application of different diagnostic methods.

## Conclusion

AD is the leading cause of dementia in older adults. However, only sixteen percent of the older adults receive regular cognitive evaluation (32). Unfortunately, the estimated extent of missed or delayed diagnosis of AD is substantial (33). Evaluation of the impairment of verbal fluency seems to have crucial diagnostic potential in the early identification of AD. Visuospatial abilities have been found to be impaired in AD even in preclinical stages and are considered to hold diagnostic potential (9, 34). Furthermore, they might have a potential role in the assessment of progression of cognitive decline since they follow linear decline among the disease course, so testing visuospatial skill might be ideal in the validation phase of drug trials.



## Author contributions

| Name | Location | Role | Contribution |
|---|---|---|---|
| Dalida Borbala Berente | Semmelweis University, Budapest | Author | She was responsible for data management and the conduction of statistical analysis. She contributed to the writing of the manuscript. |
| Anita Kamondi | National Institute of Mental Health, Neurology and Neurosurgery, Budapest | Author | She was involved in the recruitment of patients, and in the design of the study protocol. She contributed to the correction of the manuscript. |
| Andras Attila Horvath | National Institute of Mental Health, Neurology and Neurosurgery, Budapest | Author | He performed neuropsychological assessments, evaluated the results and concluded the major findings. He contributed to the writing of the manuscript. |

## Funding

Our study was supported by National Brain Research Program I, II (KTIA_NAP_13-1-2013-0001; 2017-1.2.1-NKP-2017-00002), Hungarian Scientific Research Fund 2019 of the

National Research, Development and Innovation Office (PD- 132652), Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences (bo_78_20_2020), New National Excellence Program of the National Research, Development and Innovation Office (ÚNKP-20-5-SE-16). This is an EU Joint Programme- Neurodegenerative Disease Research (JPND) project. The project is supported through the following funding organization under the aegis of JPND- www.jpnd.eu (National Research, Development and Innovation Office, Hungary, *2019*-2.1.7-ERA-NET-2020-00006*).*

## Competing Interests

The authors declare no competing interests.

## Data availability

The data that support the findings of this study and not presented in this article are available on request from the corresponding author.

## References

1. C., P., World Alzheimer Report 2018 - The state of the art of dementia research: New frontiers. *London: Alzheimer's Disease International*, 2018.
2. Reitz, C., C. Brayne, and R. Mayeux, Epidemiology of Alzheimer disease. *Nature reviews. Neurology*, 2011. 7**,**(3): p. 137-152 DOI: 10.1038/nrneurol.2011.2.
3. Braak, H. and E. Braak, Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathologica*, 1991. 82**,**(4): p. 239-59 DOI: 10.1007/bf00308809.
4. Mistridis, P., S. Krumm, A. U. Monsch, M. Berres, and K. I. Taylor, The 12 Years Preceding Mild Cognitive Impairment Due to Alzheimer's Disease: The Temporal Emergence of Cognitive Decline. *Journal of Alzheimer's Disease*, 2015. 48**,**(4): p. 1095-107 DOI: 10.3233/jad-150137.
5. Association, A. P., *Diagnostic and statistical manual of mental disorders*. Vol. 5th ed 2013: American Psychiatric Association Publishing.
6. Förstl, H. and A. Kurz, Clinical features of Alzheimer's disease. *European archives of psychiatry and clinical neuroscience*, 1999. 249**,**(6): p. 288-290 DOI: 10.1007/s004060050101.
7. Jack, C. R., Jr., D. A. Bennett, K. Blennow, M. C. Carrillo, B. Dunn, S. B. Haeberlein et al., NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, 2018. 14**,**(4): p. 535-562 DOI: 10.1016/j.jalz.2018.02.018.
8. Collie, A. and P. Maruff, The neuropsychology of preclinical Alzheimer's disease and mild cognitive impairment. *Neuroscience and biobehavioral reviews*, 2000. 24**,**(3): p. 365-74 DOI: 10.1016/s0149-7634(00)00012-9.
9. Salimi, S., M. Irish, D. Foxe, J. R. Hodges, O. Piguet, and J. R. Burrell, Can visuospatial measures improve the diagnosis of Alzheimer's disease? *Alzheimer's & dementia (Amsterdam, Netherlands)*, 2018. 10: p. 66-74 DOI: 10.1016/j.dadm.2017.10.004.
10. Quental, N. B., S. M. Brucki, and O. F. Bueno, Visuospatial function in early Alzheimer's disease--the use of the Visual Object and Space Perception (VOSP) battery. *PLoS One*, 2013. 8**,**(7): p. e68398 DOI: 10.1371/journal.pone.0068398.
11. Ghanavati, E., M. A. Salehinejad, V. Nejati, and M. A. Nitsche, Differential role of prefrontal, temporal and parietal cortices in verbal and figural fluency: Implications for the supramodal contribution of executive functions. *Scientific Reports*, 2019. 9**,**(1): p. 3700 DOI: 10.1038/s41598-019-40273-7.

12. Melrose, R., O. Campa, D. Harwood, S. Osato, M. Mandelkern, and D. Sultzer, The neural correlates of naming and fluency deficits in Alzheimer's disease: An FDG-PET study. *International journal of geriatric psychiatry*, 2009. 24: p. 885-93 DOI: 10.1002/gps.2229.
13. McKhann, G. M., D. S. Knopman, H. Chertkow, B. T. Hyman, C. R. Jack, Jr., C. H. Kawas et al., The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia*, 2011. 7**,**(3): p. 263-9 DOI: 10.1016/j.jalz.2011.03.005.
14. Duara, R., D. A. Loewenstein, E. Potter, J. Appel, M. T. Greig, R. Urs et al., Medial temporal lobe atrophy on MRI scans and the diagnosis of Alzheimer disease. *Neurology*, 2008. 71**,**(24): p. 1986-92 DOI: 10.1212/01.wnl.0000336925.79704.9f.
15. Stachó, L., R. Dudás, R. Ivády, and G. J. P. H. Kothencz, és Janka Z.(2003). Addenbrooke's Kognitív Vizsgálat: a magyar változat kifejlesztése. *Psychiatria Hungarica*. 18**,**(4): p. 226-240.
16. Dudas, R. B., G. E. Berrios, and J. R. Hodges, The Addenbrooke's cognitive examination (ACE) in the differential diagnosis of early dementias versus affective disorder. *The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry*, 2005. 13**,**(3): p. 218-26 DOI: 10.1176/appi.ajgp.13.3.218.
17. Mathuranath, P. S., P. J. Nestor, G. E. Berrios, W. Rakowicz, and J. R. Hodges, A brief cognitive test battery to differentiate Alzheimer's disease and frontotemporal dementia. *Neurology*, 2000. 55**,**(11): p. 1613-20 DOI: 10.1212/01.wnl.0000434309.85312.19.
18. Kramer, S. I. and B. V. Reifler, Depression, dementia, and reversible dementia. *Clinics in geriatric medicine*, 1992. 8**,**(2): p. 289-297 DOI: 10.1016/s0749-0690(18)30480-4.
19. Seignourel, P. J., M. E. Kunik, L. Snow, N. Wilson, and M. Stanley, Anxiety in dementia: a critical review. *Clinical psychology review*, 2008. 28**,**(7): p. 1071-82 DOI: 10.1016/j.cpr.2008.02.008.
20. de Boer, C., F. Mattace-Raso, J. van der Steen, and J. J. Pel, Mini-Mental State Examination subscores indicate visuomotor deficits in Alzheimer's disease patients: A cross-sectional study in a Dutch population. *Geriatrics & gerontology international*, 2014. 14**,**(4): p. 880-5 DOI: 10.1111/ggi.12183.
21. Hodges, J. R. and A. J. Larner, *Addenbrooke's Cognitive Examinations: ACE, ACE-R, ACE-III, ACEapp, and M-ACE*, in *Cognitive Screening Instruments*. 2017. p. 109-137.
22. Suh, G. H., Y. S. Ju, B. K. Yeon, and A. Shah, A longitudinal study of Alzheimer's disease: rates of cognitive and functional decline. *International journal of geriatric psychiatry*, 2004. 19**,**(9): p. 817-24 DOI: 10.1002/gps.1168.
23. Wilkosz, P. A., H. J. Seltman, B. Devlin, E. A. Weamer, O. L. Lopez, S. T. DeKosky et al., Trajectories of cognitive decline in Alzheimer's disease. *International psychogeriatrics*, 2010. 22**,**(2): p. 281-90 DOI: 10.1017/s1041610209991001.
24. Baudic, S., G. D. Barba, M. C. Thibaudet, A. Smagghe, P. Remy, and L. Traykov, Executive function deficits in early Alzheimer's disease and their relations with episodic memory. *Archives of Clinical Neuropsychology*, 2006. 21**,**(1): p. 15-21 DOI: 10.1016/j.acn.2005.07.002.
25. Sperling, R. A., B. C. Dickerson, M. Pihlajamaki, P. Vannini, P. S. LaViolette, O. V. Vitolo et al., Functional alterations in memory networks in early Alzheimer's disease. *Neuromolecular medicine*, 2010. 12**,**(1): p. 27-43 DOI: 10.1007/s12017-009-8109-7.
26. Murphy, K., J. Rich, and A. Troyer, Verbal fluency patterns in amnestic mild cognitive impairment are characteristic of Alzheimer's type dementia. *Journal of the International Neuropsychological Society : JINS*, 2006. 12: p. 570-4 DOI: 10.1017/S1355617706060590.
27. Clark, L., M. Gatz, L. Zheng, Y.-L. Chen, C. McCleary, and W. Mack, Longitudinal Verbal Fluency in Normal Aging, Preclinical, and Prevalent Alzheimer's Disease. *American Journal of Alzheimer's Disease and Other Dementias*, 2009. 24: p. 461-8 DOI: 10.1177/1533317509345154.

28. Gomez, R. G. and D. A. White, Using verbal fluency to detect very mild dementia of the Alzheimer type. *Archives of Clinical Neuropsychology*, 2006. 21**,**(8): p. 771-5 DOI: 10.1016/j.acn.2006.06.012.
29. Pasquier, F., F. Lebert, L. Grymonprez, and H. Petit, Verbal fluency in dementia of frontal lobe type and dementia of Alzheimer type. *Journal of neurology, neurosurgery, and psychiatry*, 1995. 58: p. 81-4 DOI: 10.1136/jnnp.58.1.81.
30. Monsch, A. U., M. W. Bondi, N. Butters, D. P. Salmon, R. Katzman, and L. J. Thal, Comparisons of verbal fluency tasks in the detection of dementia of the Alzheimer type. *Archives of neurology*, 1992. 49**,**(12): p. 1253-8 DOI: 10.1001/archneur.1992.00530360051017.
31. Henry, J. D., J. R. Crawford, and L. H. Phillips, Verbal fluency performance in dementia of the Alzheimer's type: a meta-analysis. *Neuropsychologia*, 2004. 42**,**(9): p. 1212-22 DOI: 10.1016/j.neuropsychologia.2004.02.001.
32. 2019 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, 2019. 15**,**(3): p. 321-387 DOI: https://doi.org/10.1016/j.jalz.2019.01.010.
33. Bradford, A., M. E. Kunik, P. Schulz, S. P. Williams, and H. Singh, Missed and delayed diagnosis of dementia in primary care: prevalence and contributing factors. *Alzheimer disease and associated disorders*, 2009. 23**,**(4): p. 306-314 DOI: 10.1097/WAD.0b013e3181a6bebc.
34. Hawkins, K. M. and L. E. Sergio, Visuomotor impairments in older adults at increased Alzheimer's disease risk. *Journal of Alzheimer's Disease*, 2014. 42**,**(2): p. 607-21 DOI: 10.3233/jad-140051.


## Tables

Table 1. Demographic and clinical data of participants.
Statistical tests applied were Chi-square for sex, ANOVA for parametric and Kruskal-Wallis for non-parametric statistics. One-way ANOVA analysis was used for between-group differences in memory. Kruskal-Wallis test was used for between-group differences in orientation, attention verbal fluency, language and visuospatial abilities. SD: standard deviation; MMSE: Mini-Mental State Examination IQ1-IQ3: interquartile range

| Parameter | Total | Group 0 | Group 1 | Group 2 | Group 3 | p-value |
|---|---|---|---|---|---|---|
| Participants (n) | 155 | 45 | 36 | 44 | 30 | - |
| Female, n (%) | 78 (50,3%) | 29 (64,4%) | 13 (36,11%) | 19 (43,2%) | 17 (56,7%) | 0.936 |
| Age (years) mean±SD | 71,8±7,1 | 68,6±7,4 | 70,7±7,4 | 74,1±6,2 | 74,6±5,4 | <0,001 |
| Age at disease onset (years) mean±SD | 70,2±6,4 | - | 69,2±7,3 | 71,1±6,2 | 70,0±5,6 | 0,43 |
| Education (years) median ratio (IQ1-IQ3) | 12,0 (12,0-17,0) | 17,0 (12,0-17,0) | 12,0 (12,0-16,5) | 12,0 (12,0-17,0) | 12,0 (10,0-15,0) | <0,001 |
| Disease duration (years) median ratio (IQ1-IQ3) | 3,0 (2,0-4,0) | - | 1,0 (1,0-2,0) | 3,0 (3,0-3,0) | 5,0 (4,0-5,0) | <0,001 |
| ACE total score median ratio (IQ1-IQ3) | 72,0 (59,0-88,0) | 94,0 (91,0-96,0) | 72,0 (67,3-78,0) | 66,5 (55,0-74,3) | 50,0 (45,8-57,3) | <0,001 |
| VLOM median ratio (IQ1-IQ3) | 3,3 (2,9-4,0) | 2,6 (2,4-2,9) | 3,5 (3,3-4,1) | 3,5 (3,2-4,6) | 3,6 (3,3-4,7) | <0,001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| MMSE median (IQ1-IQ3) | 22,0 (17,0-28,0) | 29,0 (28,0-29,0) | 24,0 (21,3-25,0) | 19,0 (16,0-21,0) | 15,5 (12,8-18,0) | <0,001 |
| Orientation median ratio (IQ1-IQ3) | 8,0 (7,0-10,0) | 10,0 (10,0-10,0) | 8,5 (8,0-10,0) | 7,0 (6,0-8,0) | 7,0 (5,0-8,0) | <0,001 |
| Attention median ratio (IQ1-IQ3) | 7,0 (5,0-8,0) | 8,0 (8,0-8,0) | 6,0 (5,0-7,0) | 6,0 (5,0-7,0) | 5,0 (4,0-6,0) | <0,001 |
| Memory mean± SD | 21,0±4,9 | 25,1±1,8 | 21,9±3,1 | 20,5±4,4 | 14,2±3,0 | <0,001 |
| Verbal fluency median ratio (IQ1-IQ3) | 9,0 (7,0-12,0) | 13,0 (11,0-14,0) | 9,0 (8,0-10,8) | 8,5 (6,3-10,0) | 7,0 (6,0-8,0) | <0,001 |
| Language median ratio (IQ1-IQ3) | 23,0 (19,0-28,0) | 28,0 (28,0-28,0) | 24,0 (22,0-25,0) | 20,0 (17,0-22,8) | 17,5 (15,0-20,3) | <0,001 |
| Visuospatial abilities median ratio (IQ1-IQ3) | 4,0 (4,0-5,0) | 5,0 (5,0-5,0) | 4,0 (3,3-5,0) | 3,0 (2,0-3,0) | 1,0 (0,75-2,0) | <0,001 |

Table 2. Normalized ACE subscores for orientation, attention, memory, verbal fluency, language and visuospatial abilities per group.
Normalization was performed by dividing the participant's score in each cognitive domain by the highest score possible of the same domain. (eg. 5/10 in the orientation domain resulted in a normalized score of 0,5). Differences among the cognitive subscores were compared with Wilcoxon-signed ranked test. <, > indicate the statistically significant differences with the direction ($p<0.05$), while = signals unsignificant differences ($p>0.05$). SD: standard deviation, O: orientation, A: attention, M: memory, VF: verbal fluency, L: language, VS: visuospatial abilities.

| Cognitive subdomains | Descriptive statistics | Group 0 | Group 1 | Group 2 | Group 3 |
|---|---|---|---|---|---|
| Orientation | Mean | 0,98 | 0,84 | 0,68 | 0,65 |
| | SD | 0,05 | 0,13 | 0,15 | 0,13 |
| | Differences | O=A, O>M, O>VF, O<L, O>VS | O>A, O>M, O>VF, O=L, O=VS | O<A, O<M, O>VF, O=L, O>VS | O=A, O>M, O>VF, O=L, O>VS |
| Attention | Mean | 0,99 | 0,77 | 0,76 | 0,61 |
| | SD | 0,03 | 0,15 | 0,19 | 0,17 |
| | Differences | A>M, A>VF, A=L, A>VS | A=M, A>VF, A<L, A=VS | A=M A>VF, A>L, A>VS | A>M, A>VF, A=L, A>VS |
| Memory | Mean | 0,90 | 0,78 | 0,73 | 0,51 |
| | SD | 0,06 | 0,11 | 0,16 | 0,11 |
| | Differences | M=VF, M<L, M<VS | M>VF, M<L, M=VS | M>VF, M=L, M>VS | M=VF, M<L, M>VS |
| Verbal fluency | Mean | 0,87 | 0,64 | 0,60 | 0,52 |
| | SD | 0,17 | 0,15 | 0,17 | 0,15 |

| | Differences | VF<L, VF<VS | VF<L, VF<VS | VF<L, VF>VS | VF<L, VF>VS |
|---|---|---|---|---|---|
| Language | Mean | 1,00 | 0,84 | 0,70 | 0,64 |
| | SD | 0,995 | 0,08 | 0,16 | 0,14 |
| | Differences | L>VS | L=VS | L>VS | L>VS |
| Visuospatial abilities | Mean | 0,96 | 0,81 | 0,50 | 0,25 |
| | SD | 0,08 | 0,15 | 0,23 | 0,19 |

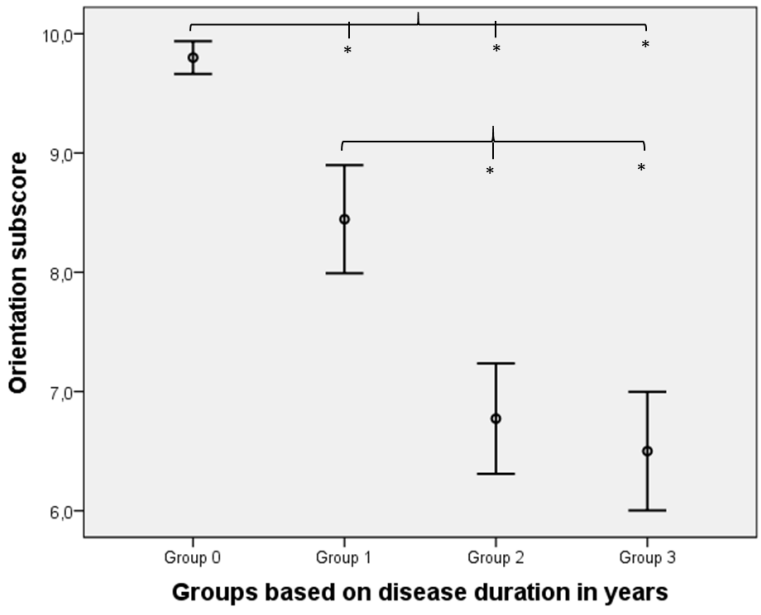
## Legends

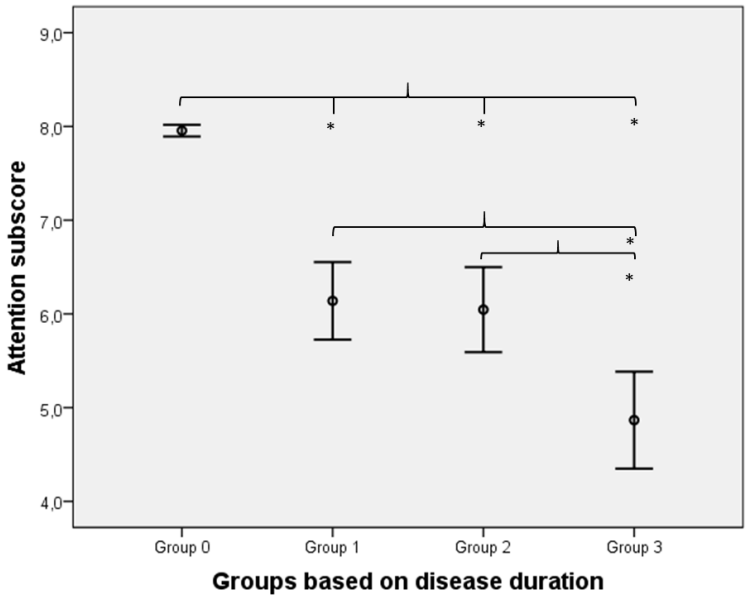
Figure 1. Between group differences for cognitive subdomains. Orientation (A) was impaired in AD from the first two years of the disease compared to healthy controls (Group 1 vs Group 0) and showed gradual decline (rapid decline in the first 4 years and remains constant afterward). Attention (B) was impaired initially (Group 0 vs Group 1), remained relatively preserved in the middle of the disease (Group 1 vs Group 2) and deteriorated again in the later phase (Group 2 vs Group 3). Memory (C) was also impaired from the first phase (Group 1 vs Group 0) but did not show prominent changes in the first 4 years of the disease (Group 1 vs Group 2), while rapid decline was detectable in the later phase (Group 2 vs Group 3). Verbal fluency (D) was highly damaged (largest difference between Group 0 and Group 1) in the first phase and did not decline further significantly. Language (E) was reduced initially (Group 1 vs Group 0) and linear decline was detectable in the first 4 years; however, changes were not so prominent at the end of the disease course (only Group 2 and Group 3 did not differ significantly). Visuospatial abilities (F) were reduced from the first phase also (Group 1 vs Group 0) and linear deterioration was highlighted (all groups differed significantly). * indicates significant differences ($p<0.01$).

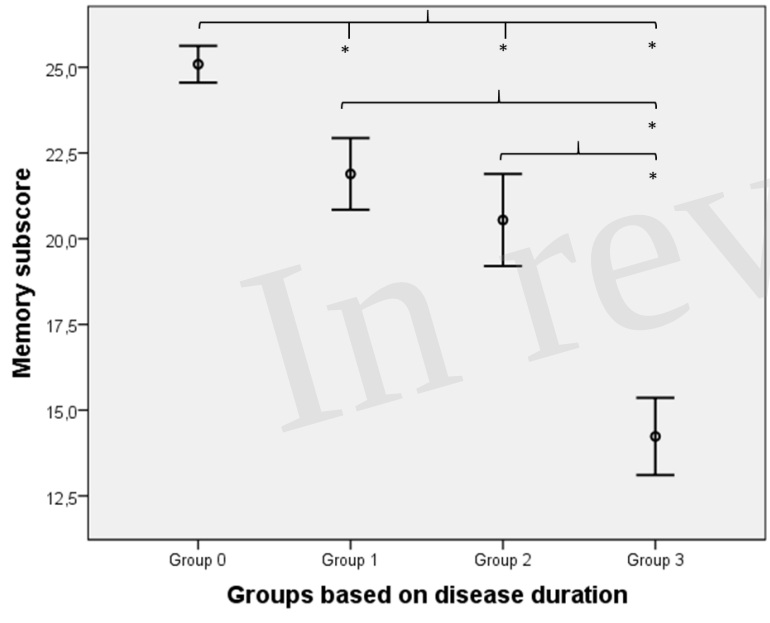
Figure 2 Correlation analysis between ACE subscores and disease duration (in years) using Spearman's rho. Significant negative correlation is present between all six subscores of orientation (A), attention (B), memory (C), verbal fluency (D), language (E) and visuospatial (F) scores (all p's$<0.05$). Visuospatial abilities associate with the steepest r line.

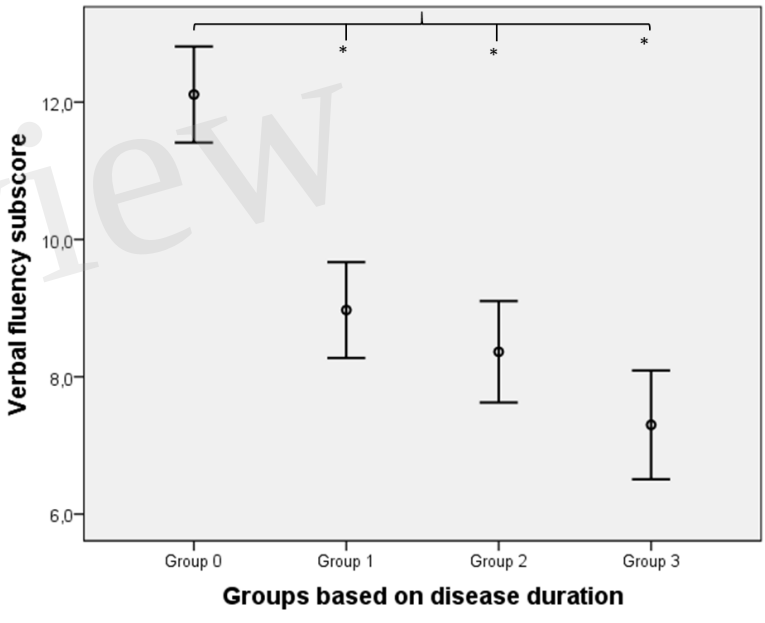
Figure 3. Within-group difference analysis for normalized ACE subscores. The contribution of verbal fluency in the cognitive maximum scores is the smallest in Group 1, suggesting prominent early impairment of this domain in the first phase of the disease. Noticeably, while the relative contribution of all cognitive domains did not change visually remarkably among the groups with various disease course, visuospatial abilities showed linear reduction in relative ratios.

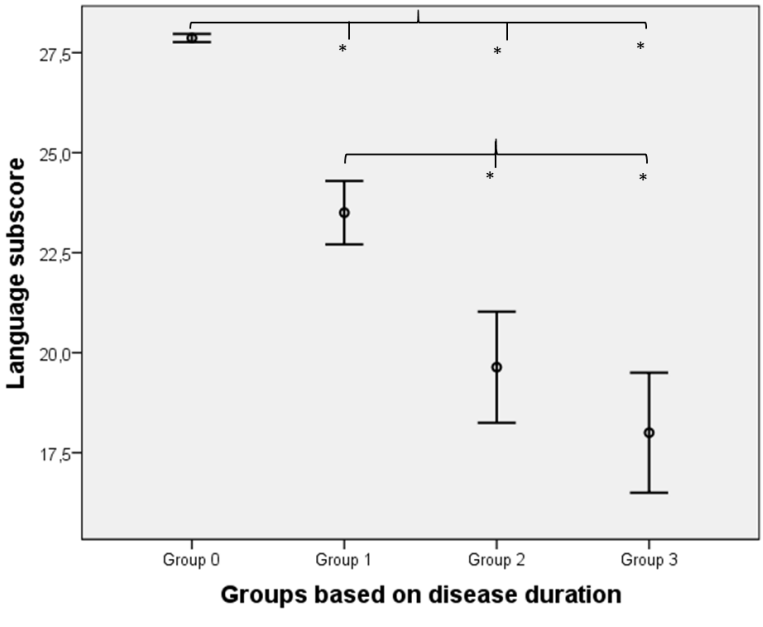
Figure 1.TIF



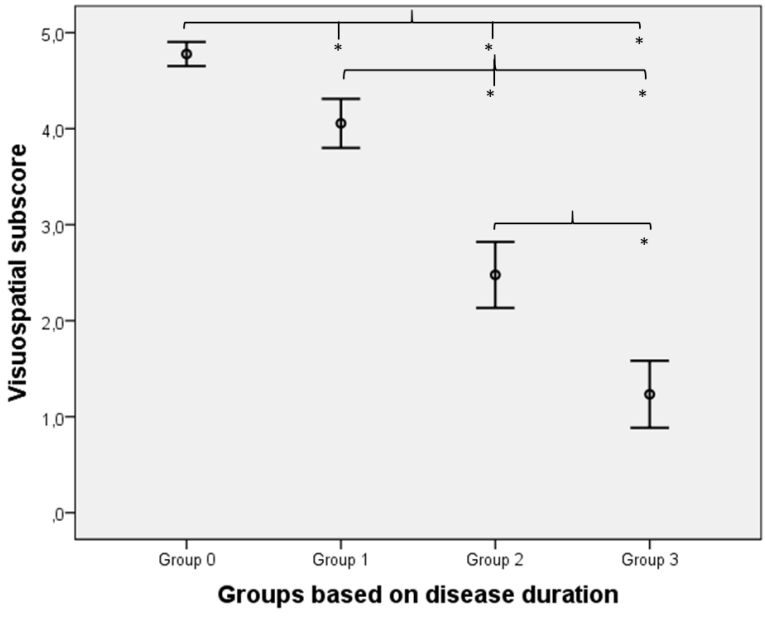
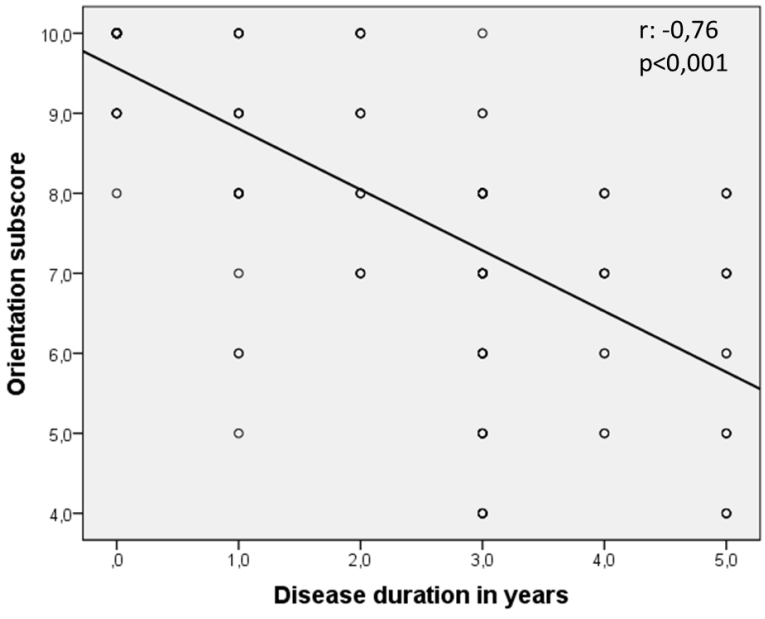
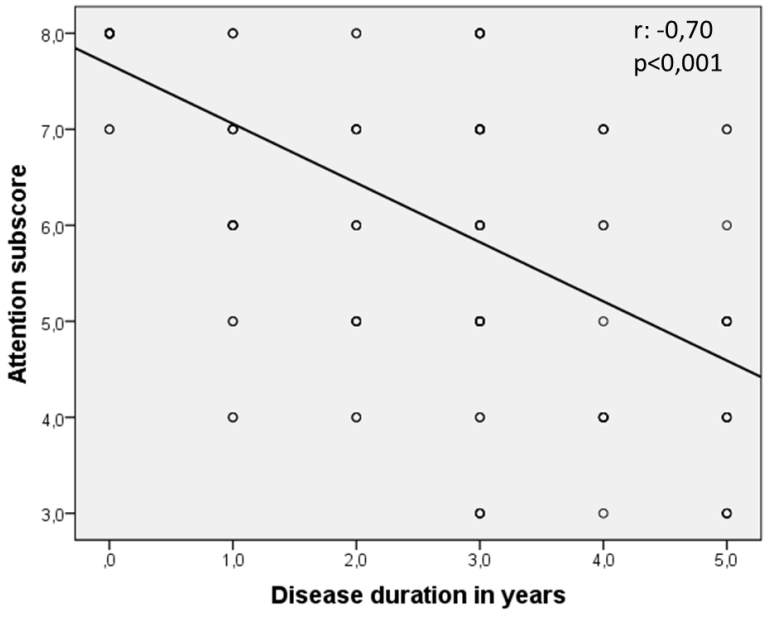
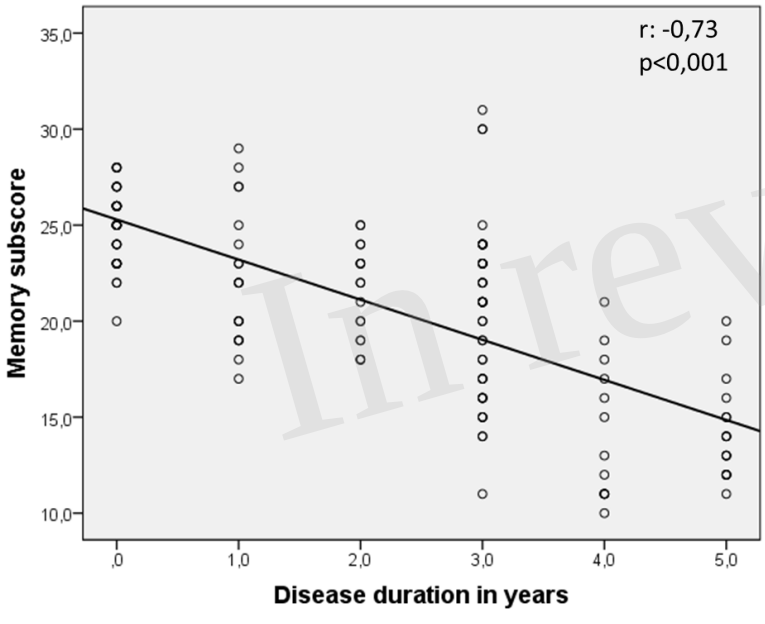
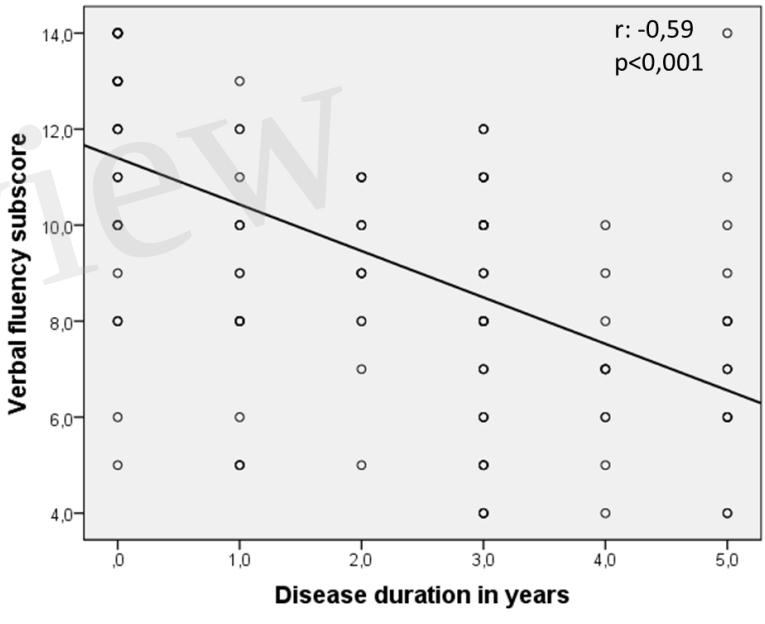



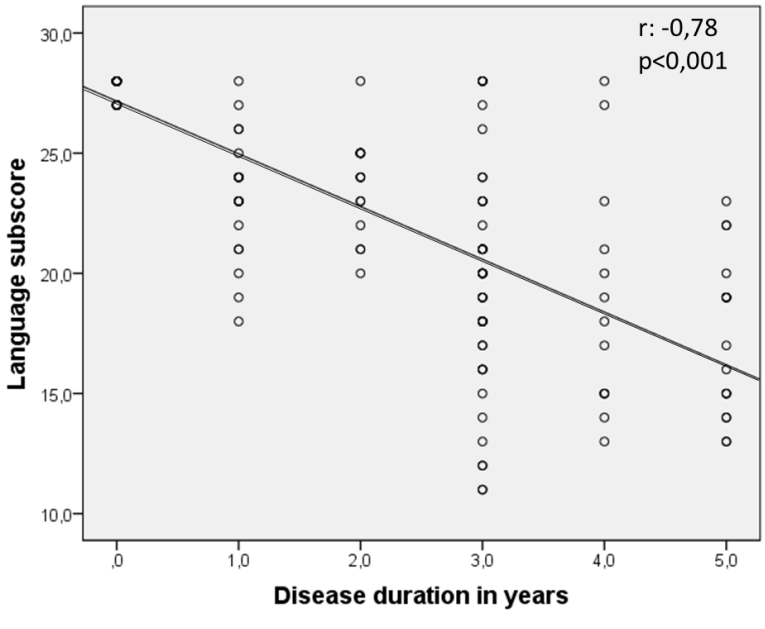
Figure 2.TIF



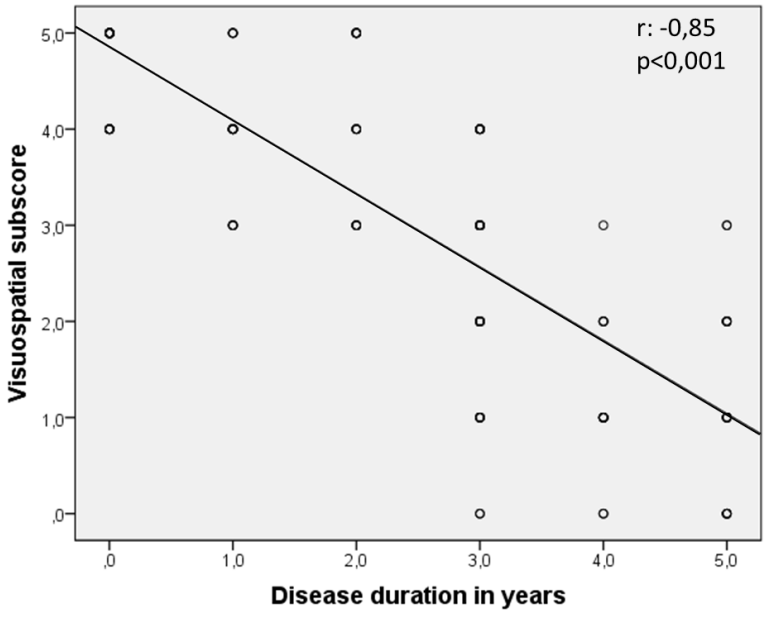



Figure 3.TIF