 doi:10.20944/preprints201905.0377.v1

Distinct subgroups of amnestic mild cognitive impairment as identified by soft independent modeling of class analogy.

Sookjaroen Tangwongchai[1], Ittipol Tawankanjanachot[2], Chavit Tunvirachaisakul[1], Thitiporn Supasitthumrong[1], Solaphat Hemrunroj[1], Phenphichcha Chuchuen[2], Thiti Snabboo[3], Andre Carvalho[4,5], Michael Maes[1]

[1] Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

[2] Department of Psychiatry, King Chulalongkorn Memorial Hospital, The Thai Red Cross Society, Bangkok, Thailand

[3] Excellence Center of Diabetes, Hormones and Metabolism, Department of medicine, Faculty of Medicine, Chulalongkorn University , Bangkok, Thailand

[4]Department of Psychiatry, University of Toronto, Toronto, ON, Canada;

[5] Centre for Addiction and Mental Health)CAMH(, Toronto, ON, Canada;

Corresponding author:

Prof. Dr. Michael Maes, M.D., Ph.D.

Department of Psychiatry

Faculty of Medicine

Patumwan

Chulalongkorn University

Bangkok 10330

Thailand

 doi:10.20944/preprints201905.0377.v1

Dr.michaelmaes@hotmail.com

https://scholar.google.co.th/citations?user=1wzMZ7UAAAAJ&hl=th&oi=sra

Sookjaroen Tangwongchai : sookjaroen@gmail.com

Ittipol Tawankanjanachot : dritthipol@gmail.com

Chavit Tunvirachaisakul : chavit.t@chula.ac.th

Thitiporn Supasitthumrong: thitiporn.s@chula.ac.th

Solaphat Hemrunroj : solaphat@hotmail.com

Phenphichcha Chuchuen : praewpsyche@gmail.com

Thiti Snabboon : thiti.S@chula.ac.th

Andre Carvalho : andre.Carvalho@camh.ca

Michael Maes: dr.michaelmaes@hotmail.com

 doi:10.20944/preprints201905.0377.v1

Abstract

Amnestic mild cognitive impairment (aMCI) is a condition characterized by mild deficits in episodic and semantic memory and learning. The conversion rate of aMCI to Alzheimer disease (AD) is significantly higher in aMCI than in the general population. The aim of this study is to examine whether aMCI is a valid diagnostic category or whether aMCI comprises different subgroups based on cognitive functions. We recruited 60 aMCI patients, 60 with AD and 61 healthy controls who completed neuropsychological tests of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD-NP) and biomarkers including serum anion gap (AGAP). Principal component analysis, support vector machine and Soft Independent Modeling of Class Analogy (SIMCA) showed that AD patients and controls were highly significantly discrimanted from each other, while patients with aMCI overlap considerably with normal controls. SIMCA showed that 68.3% of the aMCI patients were assigned to the control class (named: aMCI-HC), 15% to AD (aMCI-AD), while 16.6% did not belong to either class (aMCI-strangers). aMCI-HC subjects showed sings of very mild cognitive decline and impaired recall. aMCI-strangers showed signs of mild cognitive impairment with impaired fluency and naming. aMCI-AD cases showed a cognitive profile reminiscent of AD an increased AGAP levels. In conclusion, our SIMCA model may classify subjects afforded a clinical diagnosis of aMCI according to Petersen's criteria into three clinically relevant subgroups and help in the early detection of AD by identifying aMCI patients at risk to develop AD and those that have an AD prodrome.

Key words: dementia, AGAP, bicarbonate, cognitive function, Mild cognitive impairment, CERAD.

 doi:10.20944/preprints201905.0377.v1

Introduction

Memory disorders are common complaints that are of great concern among senior citizens around the world. Alzheimer's disease (AD) is the most common cause of dementia and accounts for around 64-90 % of the neuropathological findings in all subjects with dementia (1). AD is a progressive neuroinflammatory and neurodegenerative disorder of the brain (2, 3) and patients with that disease show a gradual decline in learning, attention, memory, executive functions and language causing impairments in social interactions and difficulties in carrying out activities of daily living (ADL) (3).

Amnestic mild cognitive impairment (aMCI) is a condition accompanied by mild deficits in episodic and semantic memory, language and learning but without decline in social interactions and ADL (4-8). MCI, as conceptualized by Petersen (4), received attention as a prodromal stage of dementia and AD, whereby MCI patients are at increased risk of developing AD (9-13). People with aMCI show an increased annual conversion rate to AD (14%) compared to the general population (1-2%) (14). Moreover, subjective memory impairment (SMI) is also an at-risk condition to develop AD (14, 15). Screening for MCI (or SMI) as an early phase of AD is, therefore, recognized as a public health priority in several countries and as a consequence reliable clinical diagnostic criteria for aMCI and neuropsychological tests to delineate the cognitive deficits are of ultimate relevance. The diagnostic criteria of MCI and AD as proposed by the Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) workgroup are based on a rigorous clinical evaluation coupled with specific laboratory investigations (to rule out reversible causes of dementia), brain neuroimaging and assessment of neuropsychological functions (16, 17).

 doi:10.20944/preprints201905.0377.v1

The Consortium to Establish a Registry for Alzheimer's, Neuropsychological Battery (CERAD-NP), is a standardized and reliable measure to differentiate both MCI and AD from health controls (18-22). For example, the CERAD-NP composite or total score shows high accuracy in distinguishing normal controls from AD and MCI patients (21, 23). Previous reports demonstrated that CERAD-NP memory measures including Wordlist Recall (WLRecall), Wordlist Memory (WLM) and Word List Recognition (WLRecognition) have a significant predictive power in differentiating AD and MCI from normal control (22, 24-26). Recently, we reported that a combination of CERAD-NP tests, namely WLRecall, WLM and Verbal Fluency Test (VFT), is useful to differentiate aMCI patients from healthy elderly controls (27) and that a lower score on the short version of the Boston Naming Test (BNT) is more predictive for AD, but not MCI (28).

The progressive cognitive decline in AD is associated with the formation of amyloid plaques, dystrophic neurites with tau protein, neuroinflammation and astrogliosis, and with neurodegenerative processes including neuronal and neuropil loss (29, 30). Genome-wide association studies show that 40% of patients with AD carry the Apolipoprotein E4 (ApoE4) allele (31), whereby the E3/E4 and especially E4/E4 genotypes considerably increase the risk of AD (32-37). On the other hand, the underlying pathophysiology of MCI remains unclear and there is no clear association between aMCI and the ApoE4 allele, whilst the conversion of aMCI to AD may occur independently of the ApoE4 genotypes (38, 39). Recently, we detected that in patients with AD and aMCI, peripheral blood biomarkers, including anion gap (AGAP) and bicarbonate levels, fasting blood glucose (FBG) and the atherogenic index of plasma (AIP) are significantly associated with deficits in semantic and episodic memory and naming ability and

 doi:10.20944/preprints201905.0377.v1

that FBG, AGAP and bicarbonate interact with ApoE4 to predict greater memory impairments (40).

Nevertheless, there are no data whether cognitive aberrations, as measured with the CERAD-NP, may model aMCI as a distinct diagnostic category, which may be discriminated from controls and AD patients or whether aMCI comprises different cognitive subgroups which may be externally validated by peripheral blood biomarkers including AGAP, FBG or AIP. Hence, the aim of the present study is to examine whether aMCI is a distinct diagnostic class based on cognitive function as assessed with the CERAD-NP or comprises subgroups with a different cognitive profile.

Subjects and Methods

Participants

In the current study we recruited 181 participants of Thai nationality, both sexes and age ranging from 55 to 90 years. All subjects with memory impairments, including aMCI and AD, were recruited at the Dementia clinic, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Healthy control subjects were community healthy aging senior club members or senior Red Cross volunteers, King Chulalongkorn Memorial Hospital. All controls and AD/aMCI patients were recruited from the same catchment area, namely Pathumwan district, Bangkok province, Thailand.

The AD diagnosis was made using criteria of the National Institute of Neurological and Communication Disorders and Stroke/AD and Related Disorders Association (NINCDS-ADRDA) (41) In addition, we included only patients with a) a score on the Thai Clinical Dementia Rating Scale (CDR) between 1 and 2; b) a score on the Thai Mini-Mental State

 doi:10.20944/preprints201905.0377.v1

Examination (MMSE-Thai) (42) between 10 and 23; and c) impaired ADL as assessed using the Blessed Dementia Scale (43, 44). aMCI patients presented with subjective memory complaints and were diagnosed using Petersen's Criteria. (4). Subjective memory complaints were assessed using the question "do you feel that your memory is becoming worse" (14, 15). Additional inclusion criteria for aMCI patients were a Thai MMSE score > 23 and a CDR score equaling 0.5. Controls were only included if they did not show subjective memory complaints and when they had a CDR score of 0 and a Thai MMSE > 23. Consequently, subjects were divided into three study groups, namely 60 AD, 60 aMCI and 61 normal controls.

We considered the following exclusion criteria for aMCI and AD patients and controls: a) medical disorders such as chronic obstructive pulmonary disease, vitamin B12 deficiency, hypothyroidism, severe heart disease (functional class II or more), cancer and chronic kidney disease; b) neurologic disorders including meningitis, encephalitis, Parkinson's disease, epilepsy, multiple sclerosis and traumatic brain injury; c) vascular and frontotemporal lobe dementia, d) other axis 1 psychiatric disorders such as affective disorders, schizophrenia and substance abuse; and e) abnormal results of blood assays including thyroid status tests and positive results for VDRL and HIV.

All participants and all guardians of patients with MCI and AD provided written informed consent prior to participation in this study. The study was conducted according to Thai and international ethics and privacy laws. Approval for the study was obtained from the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (No 359/56), which is in compliance with the International Guideline for Human Research protection as required by the Declaration of Helsinki, The Belmont Report, CIOMS Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

 doi:10.20944/preprints201905.0377.v1

Methods

All patients were assessed by a senior psychiatrist or neurologist who are specialized in dementia research. They used a semi-structured interview to score clinical history and they performed physical and neurological examinations. The CDR was used to estimate dementia staging and to confirm AD diagnosis (45). One clinical research psychologist specialized in dementia scored all participants using the CERAD-NP in a validated translation for use in a Thai population (27, 35). She was blinded from the CDR and clinical diagnosis. We scored 6 neuropsychological subdomains, namely the Modified Boston Naming Test (BNT), to probe visual naming and confrontational word retrieval; Verbal fluency Test (VFT), to probe fluency and semantic memory; the Word List Memory (WLM), to probe verbal episodic memory or immediate working memory; the Word List Recall (WLRecall) to probe delayed recall and verbal episodic memory recall, the Word List Recognition (WLRecognition) to probe verbal episodic memory-discriminability; and the Constructional Praxis test, to probe visuo-constructive abilities (18, 19). The total sum on the CERAD-NP was computed. In addition, the same research psychologist measured the Mini-Mental State Examination (MMSE) in a validated Thai translation (MMSE Thai 2002, MMSE) (42, 46). This test probes concentration, orientation, language, memory and praxis. The psychologist also measured the CERAD Blessed Dementia Scale (BDS), to probe Activities of Daily Living (ADL); the Short Blessed Test (SBT), to assess memory and concentration; and the CERAD Behavior Rating Scale for Dementia (BRSD) including depressive symptoms, inertia, vegetative symptoms, irritability/aggression, behavioral dysregulation and psychotic symptoms subscale scores. The total BRSD score was computed.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

Axis-1 DSM-IV-TR criteria were used to make the diagnosis of axis 1 psychiatric diagnosis and exclude subjects accordingly.

In addition, we performed MRI brain scans (1.5T MRI scans) and collected blood in all participants to assay thyroid function test (free T3 and T4, TSH), liver function tests (ALT, AST), serum B12 levels, complete blood count, lipid profile including total cholesterol, high density lipoprotein and low density lipoprotein cholesterol, kidney function tests including blood urea nitrogen and creatinine kinase, and serum uric acid. These blood test were performed in the Central Laboratory, King Chulalongkorn Memorial Hospital, an accredited laboratory conforming to ISO 15189 standards.

Assays

Fasting blood was sampled at 8.00 a.m from healthy controls and aMCI/AD patients for the assay of ApoE genotypes and selected biochemical measurements. This study used 3 mL clotted blood (serum), centrifuged at 1,000 g during 5 minutes and used the Architect C8000 (Abbott Laboratories, Abbott Park, Illinois, USA) to assay the following parameters: blood electrolytes (Sodium, Potassium and Chloride) measured by indirect ion selective electrode (CV 1.3%, 1.4% and 1.2%, respectively) and used to compute anion gap. Blood carbon dioxide was assayed using the PEP carboxylase method with a CV of 5.9%. Plasma glucose levels were measured by Hexakinase/ G-6-PDH technique with inter-assay coefficients of variability (CV) of 2.0%.

Genomic DNA was extracted from peripheral blood leukocytes by standard procedures with a DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). DNA was amplified by using two primers, 5'-ACAGAATTCGCCCCGGCCTGGTACACAC-3' and 5'-

 doi:10.20944/preprints201905.0377.v1

TAAGCTTGGCACGGCTGAAGGA-3'. Each amplification reaction contained 1 µg of leukocyte DNA, 1 pmol/µl of each primer, 10 % dimethyl sulfoxide, and 0.025 units/pl of *Taq* polymerase in a final volume of 30 µl. Each reaction mixture was heated at 95 °C for 5 min followed by 40 cycles of 95 °C for 60 s, 65 °C for 80 s and 72 °C for 80 s with a final extension at 72 °C for 7 min. The PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, USA) according to the protocols supplied by the manufacturer, and sent for direct sequencing to Macrogen Inc., Seoul, South Korea. We defined APOE genotype groups including APOE2 (E2/E2 or E3/E2 genotypes); b) APOE3 (including those carrying E3/E3 genotypes), and APOE4 (E4/E3 or E4/E4 genotypes). In the present study, we used the ApoE4 genotype group, denoted as any E4 allele, comprising participants carrying E4/E4, E3/E4 and E2/E4. Indeed, one E4 copy (especially E3/E4) increases risk for AD, while two E4 copies (E4/E4) increase risk considerably (36, 37)

Statistics

We used analysis of variance (ANOVA) to check differences in continuous variables between study groups and analysis of contingence tables (X^2-tests) to assess associations between nominal variables. We used univariate and multivariate GLM analysis with CERAD-NP test results as dependent variables and diagnosis as explanatory variable, followed by tests for between-subject variables and pairwise comparisons among group mean values. CERAD-NP test scores were z transformed and the mean z scores were disolayed in bar plots to display differences in cognitive profiles between groups. Tests were two-tailed and a p-value of 0.05 was used for statistical significance. All statistical analyses were performed using IBM SPSS windows version 25.

 doi:10.20944/preprints201905.0377.v1

Machine learning techniques.

We used joint principal component analysis (PCA) performed on the CERAD test results in all participants combined in order to display the distribution of the diagnostic classes in the multivariate space whereby the diagnostic groups are differentiated by marker colors and shapes. We used a standard deviation weighting process and a 20-fold cross-validation scheme. We also compute the percentage of variance explained by the extracted PCs. In the same 2D plot comprising the first two PCs we display Hotelling's T^2 ellipse (alpha=0.01%) to highlight outliers influencing the PC model. Critical outlier limits are based on 0.01% F-residuals and Hotelling's T2. Correlation loadings plot comprises two ellipses, the outer one indicating 100% explained variance and the inner one indicating 50% of the variance.

Classification through Support Vector Machine (SVM) was applied with radial basis function kernel (RBF SVM) and linear kernel (linear SVM) using the Unscrambler. The CERAD test results were normalized using a standard deviation weighting process and the SVM model was validated using a 10-fold cross-validation scheme. The capacity factor C was examined using different values in order to optimize the classification performance. We used two figures of merit namely the accuracy of the classification (percentage of correctly classified cases in both the calibration and validation samples) and the confusion matrix and show the classification results in a 2D scatter plot with the two best CERAD tests as axes.

Soft Independent Modeling of Class Analogy (SIMCA) was used to model the predefined classes AD and controls using PCA whereby each class model is delineated by analogous CERAD features among the subjects in the class model. The number of PCs employed to build the class models is determined by cross-validation and therefore may differ between the classes.

 doi:10.20944/preprints201905.0377.v1

PCA models of the classes are built after omitting outliers as detected in score, influence and stability plots, Hotelling's T2 vs samples plots, sample residual vs samples and through inspection of residual values / leverages. Extreme outliers are omitted as for example outliers that completely influence the model and those with increased residual values and leverage if they are influential. Cases with more extreme F-residual values and Hotelling's T^2 values will be eliminated if they also have CERAD values that are not relevant to the model. SIMCA models are developed in a training set (consisting of both AD and controls) and unclassified subjects (AD or controls belonging to a test set and aMCI cases) are classified by SIMCA into the class PC models for which they have the best similarity. Consequently, the distances of the subjects to the class models are computed (Si) as well as their leverage Hi (distance of the subject's projection to the model centre). Consequently, critical Si and Hi distance limits are computed and employed to classify subjects into the classes using F tests at a false negative ratio of α=0.05 or 0.01. Subjects belonging to the test set and unclassified subjects are projected into the class models whereby their critical limits determine the assignation to the modeled classes. Subjects may be assigned as members of a their target class, outsiders (not belonging to the target class) either aliens (controls intruding in the AD critical class limits) or stangers (subjects with high Hi or Si values) and hybrids (belonging to one or more classes). The Si/S0 vs Hi plot shows the residual standard deviation (relative distance of the subjects to the class model) as well as Hi. The class membership limits allow to classify subjects: when they fall within the class limits subjects belong to the target class and are therefore authenticated, whereas when they fall outside the limits they are outsiders. This plot also allows to detect aliens, namely members of another class that intrude into the target class critical limits. The most important figures of merit used in the present study are: a) the model-to-model distance, which indicates

 doi:10.20944/preprints201905.0377.v1

the strength of intergroup separation, whereby a distance between 0 and 3 indicates no real differences between the classes, a distance > 3 indicates that the class models are well discriminated and distances > 20 indicate that the models are strongly separated. b) The discrimination power of the CERAD tests indicating the impact of each CERAD probe to discriminate the class models from each other.

Results.

GLM analysis

Table 1 shows the socio-demographic data as well as CERAD measurements in normal controls, AD and aMCI patients. **Figure 1** shows the mean (SE) values of the z scores of the CERAD tests and the total CERAD score in aMCI and AD patients and healthy controls. Multivariate GLM analysis adjusted for age, sex and education shows that diagnosis has a highly significant effect (partial eta squared effect size=0.529) on the CERAD tests ($F=31.80$, $df=12/340$ $p<0.001$), while tests for between-subject effects show highly significant effects on all tests scores (with effect sizes between 0.728 for WLRecall and 0.161 for constructional praxis). Pairwise comparisons show significant differences in VFT, BNT, WLM, WLRecall and CERAD total between the three subgroups and that all tests scores significantly decreased from controls → aMCI → AD. Constructional praxis, and WLRecognition were lower in AD than in controls and aMCI, without any differences between controls and aMCI.

Principal component analysis

Figure 2 shows a first PC plot (PC1 vs PC2) performed on controls and AD patients only. Both PCs explain together 88% of the variance and therefore the loadings on both PCs can

 doi:10.20944/preprints201905.0377.v1

be interpreted accurately. This figure shows that AD patients cluster at the right-hand side of the PC plot and that controls cluster at the left-hand side, while there is a large street (boundaries) between both groups.

Figure 3 shows the same PC plot but now with inclusion of the aMCI patients. It can be seen that the latter fill in the gap between the controls and aMCI patients. The distribution of the subjects in this 2D plot indicates a continuum of cognitive dysfunction from controls to AD patients. **Figure 4** shows the correlation loadings of the CERAD test scores on both PCs indicating that VFT, WLM, WLRecall, WLrecognition and CERAD total strongly contribute to the differentiation of the classes along PC1, while constructional praxis and BNT contribute to the variability on PC1 but also PC2.

Support Vector Machine

Figure 5 shows the results of SVM with linear kernel performed on the AD patients and controls. This SVM delineated 3 support vectors, 1 control and 2 AD patients. The confusion matrix shows that all patients and controls were correctly classified and that SVM yields a classification training and validation accuracy of 100%. Figure 5 shows a plot of the classification results with CERAD total score and WLM as input variables. We have rerun the analysis with inclusion of the aMCI patients (SVM with linear kernel performed on the three groups) and found that the training accuracy was 80.7% and the validation accuracy 76.2% and that this SVM model used 88 support vectors. We also examined the SVM (linear kernel) accuracy separating HC from aMCI and found a training accuracy of 75.2% and a validation accuracy of 71.9%. The separation of aMCI from AD was more adequate with a training accuracy of 97.5% and a validation accuracy of 92.5%.

 doi:10.20944/preprints201905.0377.v1

Soft Independent Modeling of Class Analogy (SIMCA)

In order to build a SIMCA model separating AD from HC, we used a training set comprising 50% of the AD and 50% of the control subjects. The test set comprised the remaining 50% controls and AD patients, whereas all aMCI cases were subsequently projected into the AD and HC PCA models. When building the two PCA models surrounding the HC and AD classes we deleted two healthy controls and 1 AD patient from the PCA models (as statistical outliers) and we modeled both classes using 6 PCs. The model-to-model distance was 92.68 indicating a very accurate discrimination of both classes, while all CERAD variables showed significant modeling power for AD and HC. **Figure 6** shows the discrimination power of the CERAD features in separating AD from controls in descending order: WL Recall, WLRecognition, CERAD total, BNT, VFT, praxis and WLM. **Figure 7** shows the Si/S0 versus Hi plots and the distances of the subjects in the AD and control calibration sets to the AD PCA model (in the left-hand corner of the plot). We found that all AD patients (displayed as red dots), except two outsiders, were correctly classified as belonging to the AD class, while all controls (displayed as blue squares) were discriminated from the AD patients. As such, the sensitivity of the SIMCA model in authenticating AD subjects is 93.3% with a specificity of 100%. **Figure 8** shows the Si/S0 versus Hi plots and the distances of the calibration subjects to the PCs surrounding the controls. This plot shows that all controls, except one (see blue squares), were authenticated as belonging to the claimed HC class, while all AD patients (red dots) were classified as outsiders. Consequently, we have projected the 60 aMCI cases to both PCA models whereby aMCI patients are displayed as green triangles in figures 8 and 9. The results show that 9 aMCI cases (named aMCI-AD) intruded the critical limits of the AD class, while as many as 41 aMCI patients

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

(named aMCI-HC) intruded into the HC class (see Figure 9), while the classification results show that 10 aMCI patients (named: aMCI-strangers) did not belong to either the AD or HC classes. We have also rerun the analyses with two other sets of input variables, namely the 7 CERAD test scores combined with the Blessed ADL score or with the MMSE score. Those SIMCA analyses yielded similar results.

Figure 9 shows the CERAD features of the 3 aMCI subclasses delineated by SIMCA as compared with controls and AD. **Table 2** shows the socio-demographic data and CERAD tests results in the three aMCI subclasses as compared with AD and controls (see table 1 for mean values). The profile of the 41 aMCI-HC subjects showed a CERAD profile quite similar to that of controls. Moreover, multivariate GLM analyses followed by tests-for between subject effects and pairwise comparisons showed only a few mild differences between HC and aMCI-HC patients, namely WLRecall ($p=0.001$) and CERAD total score ($p=0.004$), while all other tests did not significantly differ between both groups. In addition, there were no significant differences in age, sex ratio and education between both groups. The group of the 10 aMCI-strangers showed a peculiar profile characterized by lower education, VFT and constructional praxis, while WLRecognition and WLRecall were better preserved. The 9 aMCI-AD subjects showed a profile characterized by lowered scores on all CERAD tests and thus resembles that of AD, although the severity of the cognitive features is significantly less pronounced than in AD. In order to detect a simpler decision rule based on one CERAD-NP test score rather than the SIMCA model, we have performed binary regression analyses with both aMCI-strangers + aMCI-AD groups versus AD as dependent variables and the CERAD-NP tests as explanatory variables. Neverthless, we could not find that one or more CERAD-NP tests reliably predicted those diagnoses with an accuracy > 87%.

 doi:10.20944/preprints201905.0377.v1

Figure 10 shows the BRSD items, BRSD total, Blessed ADL, SBT and MMSE scores in the 5 study groups and shows that those scores are significantly more disturbed in AD patients than in controls and those allocated to the three aMCI subgroups. Furthermore, no significant differences in any of these rating scale score could be established between the four non-AD subgroups. **Figure 11** shows the measurements of FBG, HCO3-, AGAP and AIP in the three new aMCI groups (compared with controls and AD patients, see values in Table 1). Univariate GLM analyses showed significantly lower HCO3- ($F=7.29$, $df=1/177$, $p=0.008$) and higher AGAP ($F=7.46$, $df=1/176$, $p=0.007$) levels in aMCI-AD patients as compared with the other subjects.

Discussion

The first major finding of this study is that, using SIMCA, PCA and SVM, patients with AD are highly significantly separated from healthy controls using CERAD-NP tests and that aMCI is an intermediate group considerably overlapping with healthy controls and less with AD. Our SIMCA results show that the top-3 most dominant features of AD are lowered scores on WLRecall, WLRecognition and BNT. As reviewed in the introduction, there are some studies examining the cognitive profile of AD versus controls showing that the CERAD total score may be used to discriminate AD patients from controls with an area under the receiving operating curve (ROC) of 0.970 (22). An area under the ROC > 0.95 when bootstrapped may indicate that an accurate prediction can be made (47,48).

Our SMV and SIMCA data show that CERAD-NP tests may be combined into a decision rule to classify patients with AD versus controls with great accuracy, namely 100% for SVM and 97.5% for SIMCA. In general, a combination of different features (such as CERAD-NP test

 doi:10.20944/preprints201905.0377.v1

results) into a multivariate decision rule will lead to a better discrimination than using one rating scale alone. In addition, when the new machine learning model is (cross-) validated and shows a validation accuracy of > 95%, the new decision rule has predictive value (47). Nevertheless, the SVM separation of aMCI patients from healthy controls yielded only a very moderate validation accuracy (71.9%). In the Seo et al. (22) study, an area under the ROC curve of 0.82 is reported for the separation of aMCI from controls with a sensitivity of 79.2% and a specificity of 85.0%. These figures of merit do not allow a predictive classification (47) of aMCI versus controls using the CERAD-NP scores. All in all, these results show that aMCI cannot be successfully discriminated from healthy controls using CERAD-NP test results as input variables.

In our study using a combination of different CERAD-NP tests we found that aMCI and AD were relatively well separated using SVM (validation accuracy: 92.5%) and SIMCA (validation accuracy of 89.17%). Using SIMCA we found that the top-3 features separating AD from aMCI are in descending order: WLRecall, WLRecognition and WLM, indicating that WLRecall and WLRecognition are the most adequate features to discriminate AD from controls and aMCI patients. In the Seo et al. (22) study, the separation of MCI versus AD using the CERAD total score yielded an area under the ROC curve of 0.86. Nevertheless, these figures of merit do not allow the SIMCA, SVM or ROC models to be used for prediction purposes (47).

Based on the accuracy of both SVM (100%) and SIMCA (97.2%) separating AD from controls we concluded that those models may be employed to classify unknown cases into AD or the control class. Nevertheless, SVM operates with the assumption that all cases should belong to one of the pre-specified classes and therefore SVM forces aMCI subjects to be classified as AD cases or controls (47). Accordingly, SVM methods miss precision because this method cannot identify cases that belong to two classes (hybrids) or do not belong to any predefined class

 doi:10.20944/preprints201905.0377.v1

(strangers) (47). In this regard, SIMCA improves precision of the identification process because this technique allows to authenticate AD patients as belonging to the presumed AD class, and to identify unknown subjects as aliens, hybrids or strangers (47). Therefore, our validated SIMCA models built using the control and AD classes may be used to identify aMCI patients.

The second major finding of our study is that aMCI as diagnosed with Petersen' criteria (4) consists of three distinct subgroups. Using CERAD-NP scores, SIMCA assigned 68.3% of the patients to the control class (named: aMCI-HC), 15% to the AD class (named: aMCI-AD), while 16.6% did not belong to either class (aMCI-strangers). It should be underscored that all our aMCI subjects scored 0.5 on the CDR Staging Dementia Instrument indicating that they suffered from very mild dementia with mild memory impairments, slight impairments in cummunity affairs without dysfunctions in personal care (45). As such, an assignment of aMCI patients to the control or AD class indicates that their cognitive profile (but not behavioral symptoms or ADL) is quite similar to that of these target groups, but does not allow to conclude that these subjects may be diagnosed as AD or controls.

The main characteristic of aMCI-HC subjects was a lowered WLRecall score but the other neurocognitive CERAD probes were quite similar to that of normal controls with a difference in total CERAD score of only 0.276 SDs. Therefore, these individuals could be diagnosed as SMI, because they show subjective memory complaints and a deficit in delayed recall (14, 15). According to these authors, SMI is an at-risk condition to develop AD (14, 15), although this effect may be confined to SMI with self-reported concerns (15). In our study subjective memory impairment was one of the criteria to diagnose aMCI although we did not measure "self-reported concerns". By inference our aMCI-HC subgroup cannot be considered to constitute an at-risk group to develop AD.

 doi:10.20944/preprints201905.0377.v1

The two other aMCI subgroups defined by SIMCA showed a much lower CERAD total score that differed cosiderably from that of controls, namely 0.901 SDs for aMCI-strangers and 1.16 SDs for the aMCI-AD cases. Increasing levels of cognitive impairment in MCI are at-risk factors of AD (15, 49), indicating that these two SIMCA subgroups may be at increased risk to the develop AD. The main characteristics of the aMCI-stranger subgroup were significantly lower scores on VFT, praxis and naming. As such this subgroup shows more selective deficits in language and fluency, semantic memory and praxis. It could be that this subgroup is a prodromal stage of semantic dementia (50), which is accompanied by loss of fluent speech and poor category fluency without loss of episodic memory. The second subgroup (aMCI-AD) is clearly a dementia prodrome as the constellation of cognitive dysfuctions observed in these patients resembles that of AD, although with less severity. Once behavioral symptoms develop or personal care deteriorates these aMCI-AD subjects will be diagnosed as AD.

Although we did not measure the Global Deterioration Scale (GDS) for Assessment of Primary Degenerative Dementia (51) it is interesting to apply this staging scale to our SIMCA aMCI subgroups. Thus, the aMCI-HC patients probably belong to GDS stage 2 or very mild cognitive decline, while aMCI-strangers probably belong to GDS stage 3 or mild cognitive decline. Subjects allocated to the aMCI-AD subgroup belong to the transition zone from stage 3 to stage 4 (moderate cognitive decline) which is an early stage dementia.

Our SIMCA findings may question the definitions of early CMI (EMCI) [a condition characterized by cognitive impairments on a standardized test, which are 1.0-1.5 SDs lower than the normative mean] and late MCI (LMCI) [a condition characterized by cognitive impairments on a standardized test, which are > 1.5 SDs lower than the normative mean] (52). In fact, while our aMCI-AD patients are allocated to the AD group based on a similar cognitive profile they

 doi:10.20944/preprints201905.0377.v1

show impairments on MMSE, SBT and CERAD total of around 1.2 SDs, but not 1.5 SDs, lower than the normative mean. In the current study we detected that a combination of different CERAD-NP features in a SIMCA model is a very adequate method to define a subgroup (aMCI-AD) of patients with a cognitive profile that is reminiscent of AD but cannot be classified as AD because ADL is still intact. Future prospective research in early AD recognition studies should therefore compare the predictive value of our SIMCA decision rule versus the case definitions of SMI, ECMI and LCMI as predictors of AD in larger study groups.

The third major finding of this study is that the SIMCA-derived aMCI-AD subgroup was externally validated by highly increased AGAP and lowered HCO3- values (but not ApoE4, FBG or AIP). Recently, we reported that increased AGAP and lowered bicarbonate levels are associated with impairments in episodic and semantic memory in AD and aMCI and that interactions between AGAP and ApoE4 increase cognitive decline whereby among ApoE4 carriers, increased AGAP values are associated with increased cognitive impairments (40). As such, the results of the current study show that increased levels of AGAP are associated with a possible dementia prodrome (aMCI-AD) and, therefore, possibly with the transition of aMCI to AD. Some previous studies reported that lowered bicarbonate levels are associated with confusion, delirium andcognitive and executive impairments (53-55). One possible explanation is that increased AGAP or lowered bicarbonate levels may have pro-inflammatory and pro-oxidative properties thereby aggravating the neuroinflammatory and neurodegenerative pathways leading to AD **(**40**)**.

In conclusion, in the current study we have delineated a SIMCA model that can be used clinically to classify subjects with aMCI into subgroups, which appear to indicate different stages of cognitive decline. The first subgroup are subjects with a very mild cognitive decline are

 doi:10.20944/preprints201905.0377.v1

differentiated from controls by SMI and lowered delayed recall. The second subgroup displays signs of mild cognitive decline with lowered fluency and naming as important features and these subjects may be at an increased risk to develop dementia. The third subgroup comprises patients in the transition zone from moderate cognitive decline to early-stage dementia and, therefore, these patients show a dementia prodrome. **Figure 12** shows clinical relevance of our SIMCA decision rule: once the clinical diagnosis of aMCI is made using Petersen's criteria, the clinician may enter the 6 CERAD-NP test results in the SIMCA program, which consequently will classify the aMCI patients in the 3 clinically relevant subgroups. As such, SIMCA may identify aMCI patients who are at risk to develop AD or show an AD prodrome. Therefore, our SIMCA diagnosis may help in the early detection of AD and as a consequence may help to reduce the transition rate from aMCI to AD by targeting relevant and modifiable pathways, including increased AGAP.

Funding

This project was supported by the Chulalongkorn University, Thai Government Fund: GRB_APS_10_57_30_08

Conflict of interest

The authors have no conflict of interest with any commercial or other association in connection with the submitted article.

Author's contributions

 doi:10.20944/preprints201905.0377.v1

All the contributing authors have participated in preparation of the manuscript. The work was designed by ST, IT, TS, SH, MM and CT. Data were collected by TS, CT, PC, ST and IT. Laboratory analyses were conducted by TS. Statistical analyses were performed by AC and MM.

References

1. Barker WW, Luis CA, Kashuba A, Luis M, Harwood DG, Loewenstein D, et al. Relative frequencies of Alzheimer disease, Lewy body, vascular and frontotemporal dementia, and hippocampal sclerosis in the State of Florida Brain Bank. Alzheimer Disease and Associated Disorders. 2002;16(4):203-212.

2. Burns A, Iliffe S. Alzheimer's disease. BMJ (Clinical research ed). 2009 Feb 5;338:b158.

3. World Health Organization: Dementia fact sheet World Health Organization; 2017 [cited 2018 8 September]. Available from: http://www.who.int/en/news-room/fact-sheets/detail/dementia.

4.Petersen RC. Mild cognitive impairment as a diagnostic entity. Journal of Internal Medicine. 2004;256(3):183-194.

5. Petersen RC, Caracciolo B, Brayne C, Gauthier S, Jelic V, Fratiglioni L. Mild cognitive impairment: a concept in evolution. Journal of Internal Medicine. 2014;275(3):214-228.

6. Neugroschl J, Wang S. Alzheimer’s Disease: Diagnosis and Treatment Across the Spectrum of Disease Severity. The Mount Sinai Journal of Medicine, New York. 2011;78(4):596-612.

7. Tarawneh R, Holtzman DM. The clinical problem of symptomatic Alzheimer disease and mild cognitive impairment. Cold Spring Harbor Perspectives in Medicine. 2012;2(5):a006148. PubMed PMID: 22553492. Pubmed Central PMCID: PMC3331682. Epub 2012/05/04. eng.

8. Jahn H. Memory loss in Alzheimer's disease. Dialogues in Clinical Neuroscience. 2013;15(4):445-54.

9. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Archives of Neurology. 1999;56(3):303-308.

10. Petersen RC, Negash S. Mild cognitive impairment: an overview. CNS Spectrums. 2008;13(1):45-53.

11. Mitchell AJ, Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort studies. Acta Psychiatrica Scandinavica. 2009;119(4):252-265.

12. Koivunen J, Scheinin N, Virta JR, Aalto S, Vahlberg T, Nagren K, et al. Amyloid PET imaging in patients with mild cognitive impairment: a 2-year follow-up study. Neurology. 2011;76(12):1085-1090.

13. Buchhave P, Minthon L, Zetterberg H, Wallin AK, Blennow K, Hansson O. Cerebrospinal fluid levels of beta-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. Archives of General Psychiatry. 2012;69(1):98-106.

14. Jessen F, Wiese B, Bachmann C, Eifflaender-Gorfer S, Haller F, Kolsch H, et al. Prediction of dementia by subjective memory impairment: effects of severity and temporal association with cognitive impairment. Archives of General Psychiatry. 2010;67(4):414-422.

15. Jessen F. Subjective and objective cognitive decline at the pre-dementia stage of Alzheimer's disease. European Archives of Psychiatry and Clinical Neuroscience. 2014;264 Suppl 1:S3-7.

16. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. 1984;34(7):939-944.

17. Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. The Lancet Neurology. 2007;6(8):734-46.

18. Morris JC, Mohs RC, Rogers H, Fillenbaum G, Heyman A. Consortium to establish a registry for Alzheimer's disease (CERAD) clinical and neuropsychological assessment of Alzheimer's disease. Psychopharmacology Bulletin. 1988;24(4):641-652.

19. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology. 1989;39(9):1159-1165.

20. Welsh K, Butters N, Hughes J, Mohs R, Heyman A. Detection of abnormal memory decline in mild cases of Alzheimer's disease using CERAD neuropsychological measures. Archives of Neurology. 1991;48(3):278-281.

21. Paajanen T, Hanninen T, Tunnard C, Mecocci P, Sobow T, Tsolaki M, et al. CERAD neuropsychological battery total score in multinational mild cognitive impairment and control populations: the AddNeuroMed study. Journal of Alzheimer's Disease 2010;22(4):1089-1097.

22. Seo EH, Lee DY, Lee JH, Choo IH, Kim JW, Kim SG, et al. Total scores of the CERAD neuropsychological assessment battery: validation for mild cognitive impairment and dementia patients with diverse etiologies. The American Journal of Geriatric Psychiatry. 2010;18(9):801-809.

23. Chandler MJ, Lacritz LH, Hynan LS, Barnard HD, Allen G, Deschner M, et al. A total score for the CERAD neuropsychological battery. Neurology. 2005;65(1):102-106.

24. Karrasch M, Sinerva E, Gronholm P, Rinne J, Laine M. CERAD test performances in amnestic mild cognitive impairment and Alzheimer's disease. Acta Neurologica Scandinavica. 2005;111(3):172-179.

25. Sotaniemi M, Pulliainen V, Hokkanen L, Pirttila T, Hallikainen I, Soininen H, et al. CERAD-neuropsychological battery in screening mild Alzheimer's disease. Acta Neurologica Scandinavica. 2012;125(1):16-23.

26. Aguirre-Acevedo DC, Jaimes-Barragan F, Henao E, Tirado V, Munoz C, Reiman EM, et al. Diagnostic accuracy of CERAD total score in a Colombian cohort with mild cognitive impairment and Alzheimer's disease affected by E280A mutation on presenilin-1 gene. International Psychogeriatrics. 2016;28(3):503-510.

27. Tunvirachaisakul C, Supasitthumrong T, Tangwongchai S, Hemrunroj S, Chuchuen P, Tawankanjanachot I, et al. Characteristics of Mild Cognitive Impairment Using the Thai Version of the Consortium to Establish a Registry for Alzheimer's Disease Tests: A Multivariate and Machine Learning Study. Dementia and Geriatric Cognitive Disorders. 2018;45(1-2):38-48.

28. Aniwattanapong D, Tangwongchai S, Supasitthumrong T, Hemrunroj S, Tunvirachaisakul C, Tawankanjanachot I, et al. Validation of the Thai version of the short Boston Naming Test (T-BNT) in patients with Alzheimer's dementia and mild cognitive impairment: clinical and biomarker correlates. Aging & Mental Health. 2018;23:1-11.

29. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Molecular Medicine. 2016;8(6):595-608. PubMed PMID: 27025652.

30. Takahashi RH, Nagao T, Gouras GK. Plaque formation and the intraneuronal accumulation of beta-amyloid in Alzheimer's disease. Pathology International. 2017;67(4):185-93.

 doi:10.20944/preprints201905.0377.v1

31. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA. 1997;278(16):1349-13456.

32. Qian J, Wolters FJ, Beiser A, Haan M, Ikram MA, Karlawish J, et al. APOE-related risk of mild cognitive impairment and dementia for prevention trials: An analysis of four cohorts. PLoS medicine. 2017;14(3):e1002254.

33. Schipper HM. Apolipoprotein E: implications for AD neurobiology, epidemiology and risk assessment. Neurobiology of Aging. 2011;32(5):778-790.

34. Riedel BC, Thompson PM, Brinton RD. Age, APOE and sex: Triad of risk of Alzheimer's disease. The Journal of Steroid Biochemistry and Molecular Biology. 2016;160:134-147.

35. Tangwongchai S, Supasitthumrong T, Hemrunroj S, Tunvirachaisakul C, Chuchuen P, Houngngam N, et al. In Thai Nationals, the ApoE4 Allele Affects Multiple Domains of Neuropsychological, Biobehavioral, and Social Functioning Thereby Contributing to Alzheimer's Disorder, while the ApoE3 Allele Protects Against Neuropsychiatric Symptoms and Psychosocial Deficits. Molecular Neurobiology. 2018;55(8):6449-6462.

36. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science (New York, NY). 1993;261(5123):921-923.

37. Roses AD. Apolipoprotein E alleles as risk factors in Alzheimer's disease. Annual Review of Medicine. 1996;47:387-400.

38. Falahati F, Ferreira D, Muehlboeck JS, Eriksdotter M, Simmons A, Wahlund LO, et al. Monitoring disease progression in mild cognitive impairment: Associations between atrophy patterns, cognition, APOE and amyloid. NeuroImage Clinical. 2017;16:418-28.

39. Jefferson AL, Beiser AS, Seshadri S, Wolf PA, Au R. APOE and mild cognitive impairment: the Framingham Heart Study. Age and Ageing. 2015 Mar;44(2):307-11.

40. Supasitthumrong T, Tunvirachaisakul C., Aniwattanapong D., Tangwongchai S., Chuchuen P., Tawankanjanachot I., Snabboon., Hemrungrojn S., Carvalho A F., Maes M. Peripheral blood biomarkers coupled with the Apolipoprotein E4 genotype are strongly associated with semantic and episodic memory impairments in elderly subjects with amestic mild cognitive impairment and Alzheimer's dementia. Preprints 2019; 2019010296 (doi: 10.20944/preprints201901.0296.v1).

41. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, 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 May;7(3):263-9.

42. Medical technology assessment project committee. The Comparison of the Test Performance Between the MMSE-Thai 2002 and the TMSE for Dementia Screening in the Elderly. Bangkok, Thailand: Thai Geriatric Medicine Institute, Ministry of Public Health; 2008.

43. Blessed G, Tomlinson BE, Roth M. The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. The British Journal of Psychiatry. 1968;114(512):797-811.

44. Blessed G, Tomlinson BE, Roth M. Blessed-Roth Dementia Scale (DS). Psychopharmacology Bulletin. 1988;24(4):705-708.

45. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology. 1993;43(11):2412-2414.

46. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-98.

47. Al-Hakeim H. A-FS, Aldujaili A., Carvalho AF., Sriswasdi S., Maes M. Development of a novel neuro-immune and opioid-association fingerprint with a cross-validated ability to identify and authenticate unknown patients with major depression: far beyond differntiation, discrimination and classification. Molecular Neurobiology, in press; 2019.

48. Alamprese C, Casiraghi E. Application of FT-NIR and FT-IR spectroscopy to fish fillet authentication. LWT-Food Science and Technology. 2015;63:720-5.

49. Gomar JJ, Bobes-Bascaran MT, Conejero-Goldberg C, Davies P, Goldberg TE, Alzheimer's Disease Neuroimaging I. Utility of combinations of biomarkers, cognitive markers, and risk factors to predict conversion from mild cognitive impairment to Alzheimer disease in patients in the Alzheimer's disease neuroimaging initiative. Archives of General Psychiatry. 2011;68(9):961-9.

50. Kertesz A, Jesso S, Harciarek M, Blair M, McMonagle P. What is semantic dementia?: a cohort study of diagnostic features and clinical boundaries. Archives of Neurology. 2010;67(4):483-489.

51. Reisberg B, Ferris SH, de Leon MJ, Crook T. Global Deterioration Scale (GDS). Psychopharmacology Bulletin. 1988;24(4):661-663.

52. Aisen PS, Petersen RC, Donohue MC, Gamst A, Raman R, Thomas RG, et al. Clinical Core of the Alzheimer's Disease Neuroimaging Initiative: progress and plans. Alzheimer's & Dementia. 2010;6(3):239-246.

 doi:10.20944/preprints201905.0377.v1

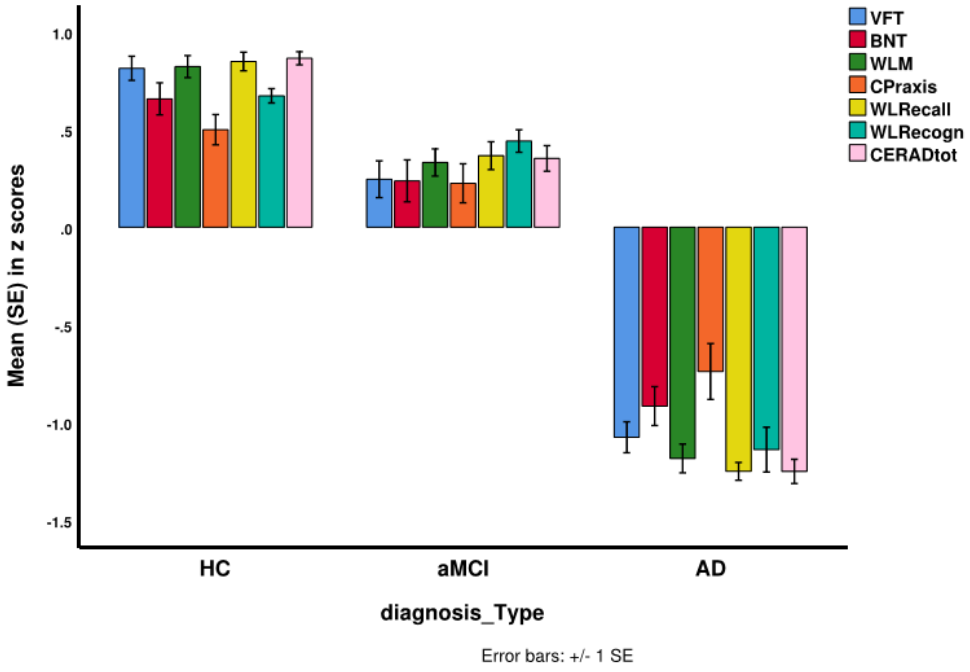
53. Dobre M, Gaussoin SA, Bates JT, Chonchol MB, Cohen DL, Hostetter TH, et al. Serum Bicarbonate Concentration and Cognitive Function in Hypertensive Adults. Clinical journal of the American Society of Nephrology : CJASN. 2018;13(4):596-603.

54. Sozio SM, McAdams-DeMarco M. The Role of Bicarbonate in Cognition: Acidosis May Be Corrosive to the Brain. Clinical journal of the American Society of Nephrology: CJASN. 2018;13(4):527-8. PubMed PMID: 29567860. Pubmed Central PMCID: 5969456.

55. Potharajaroen S, Tangwongchai S, Tayjasanant T, Thawitsri T, Anderson G, Maes M. Bright light and oxygen therapies decrease delirium risk in critically ill surgical patients by targeting sleep and acid-base disturbances. Psychiatry Research. 2018;261:21-27. PubMed PMID: 29276990.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

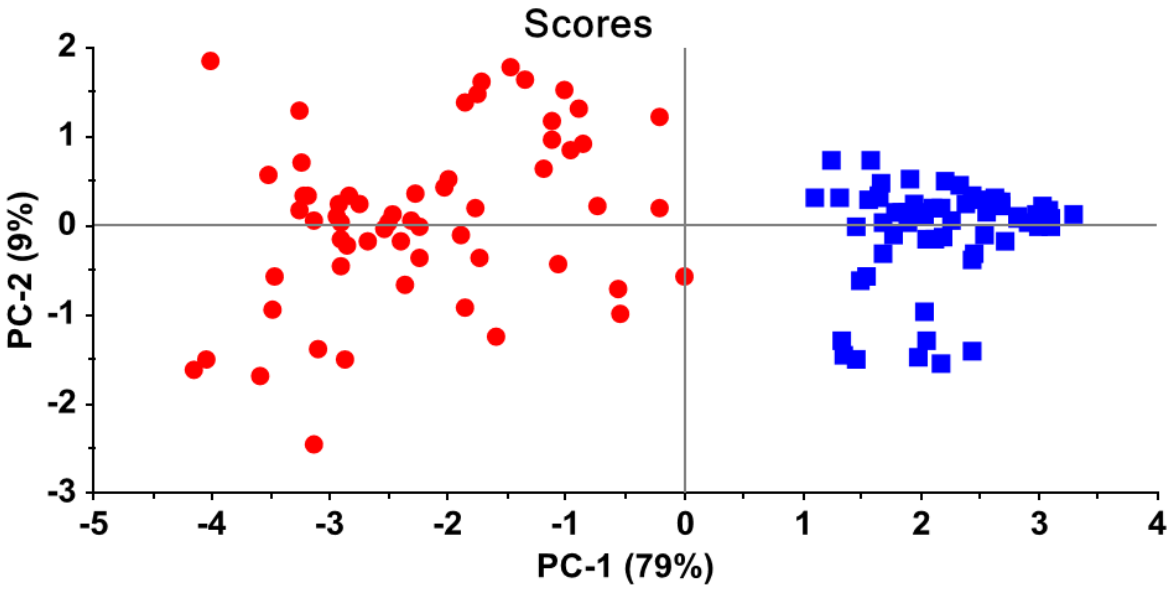
Figure 1. Mean (SE) values of the z scores of the CERAD tests and the total CERAD score (CERADtot) in patients with amnestic mild cognitive impairment (aMCI) and Alzheimer's disease (AD) and healthy controls (HC).



VFT: Verbal Fluency test; BNT: Boston Naming Test; WLM: Worl List Memory; CPraxis: Constructional praxis; WLRecall: Word List Recall; WLRecogn: Word List Recognition

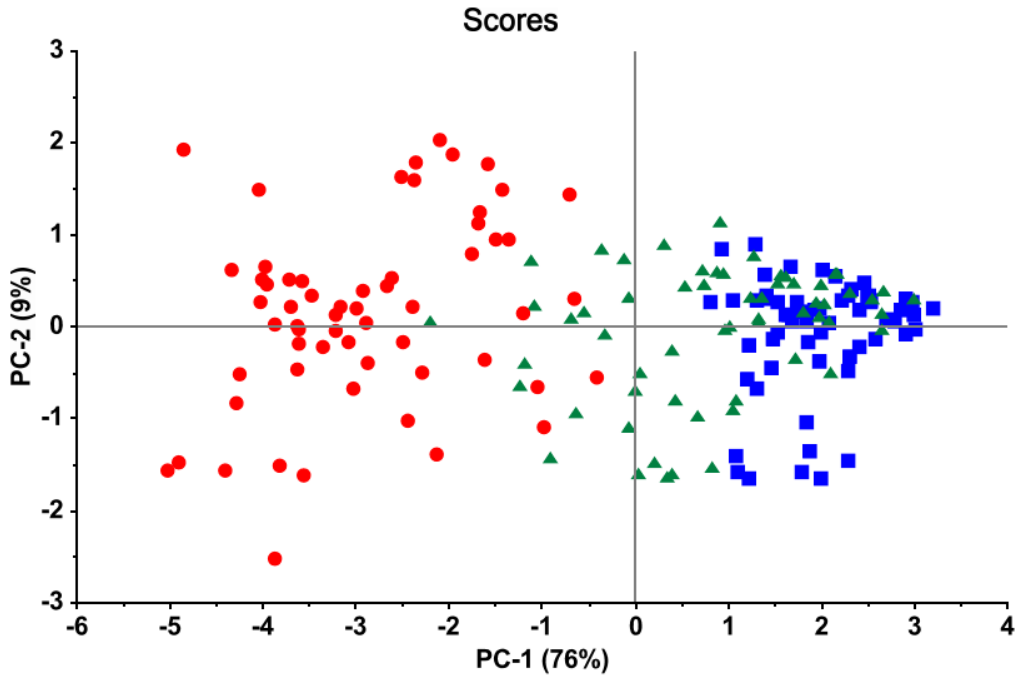
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

Figure 2. Principal Component (PC) plot (PC1 vs PC2) performed using CERAD data from controls (blue squares) and AD (red dots) patients only.



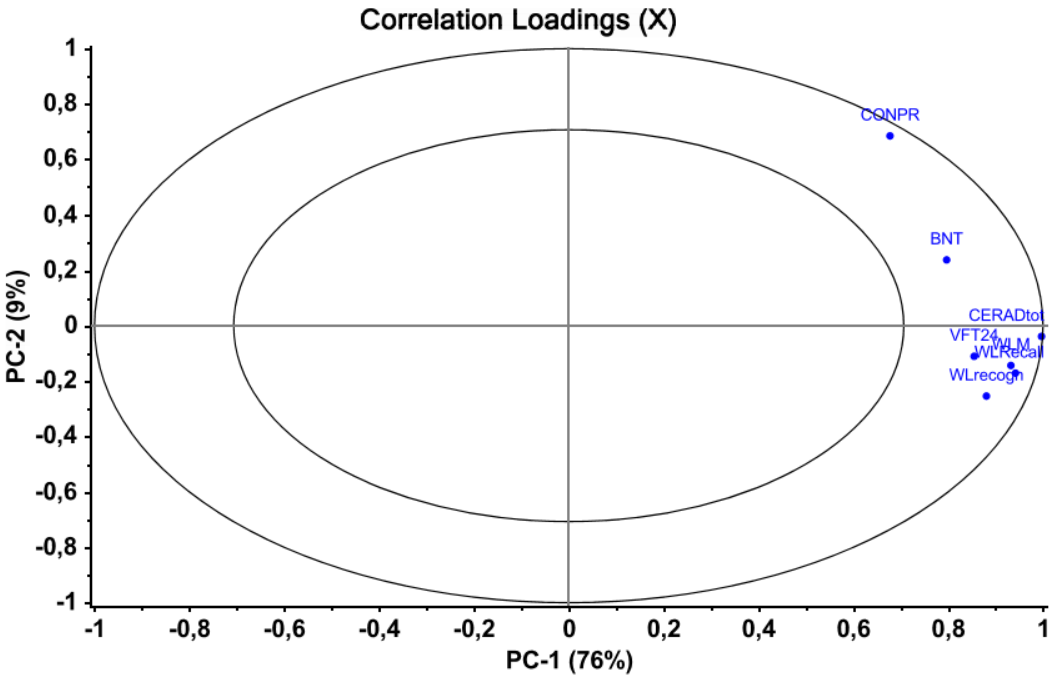
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

Figure 3. Principal Component (PC) plot using CERAD data from controls (blue squares) and AD (red dots) (see also figure 2) but now also including patients with amnestic mild cognitive impairment (green triangles).



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

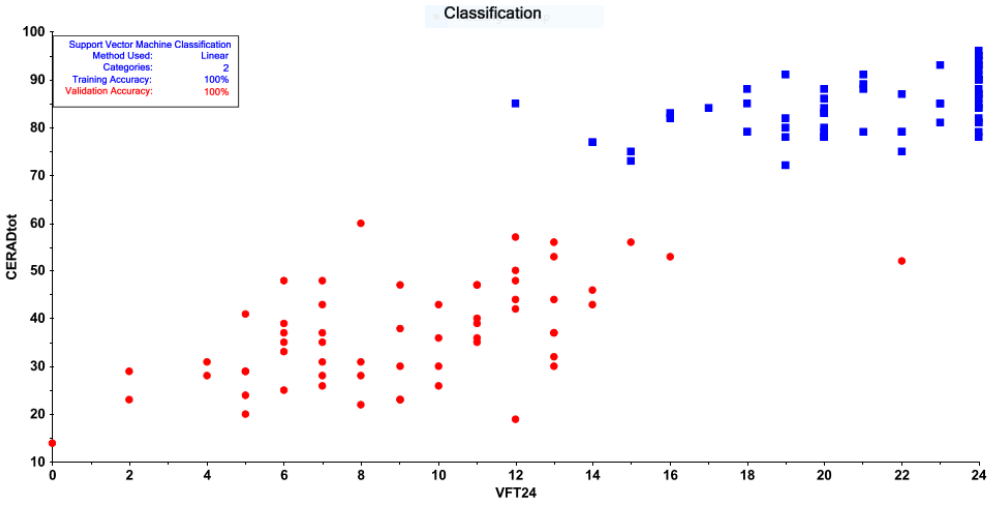
Figure 4. Correlation loadings of CERAD test scores on principal component (PC)1 and PC2.



VFT: Verbal Fluency test; BNT: Boston Naming Test; WLM: Worl List Memory; CPraxis: Constructional praxis; WLRecall: Word List Recall; WLRecogn: Word List Recognition; CERADtot: total score.

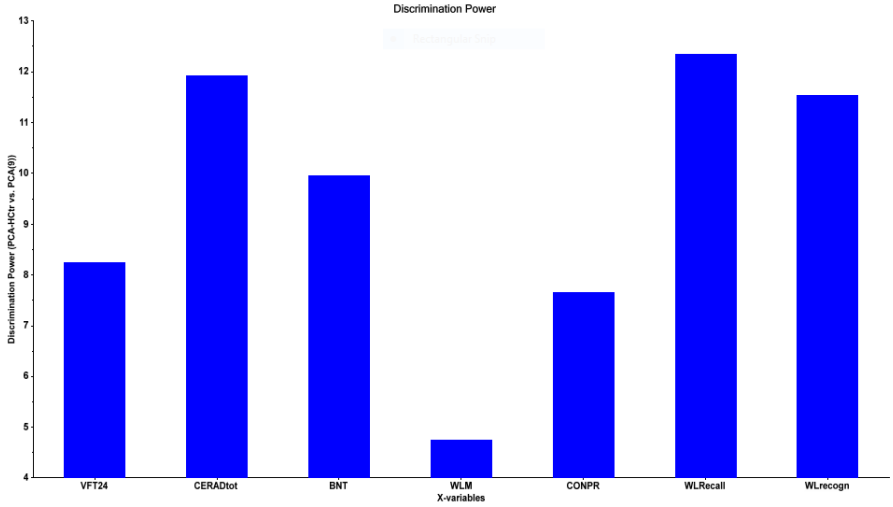
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

Figure 5. Results of Support Vector Machine with all CERAD tests as input variables. Healthy controls are shown as blue squares, Alzheimer patients as red dots. This plot shows the results of classification results with and CERAD total score (CERADtot) and Word List Memory (WLM) as input variables



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

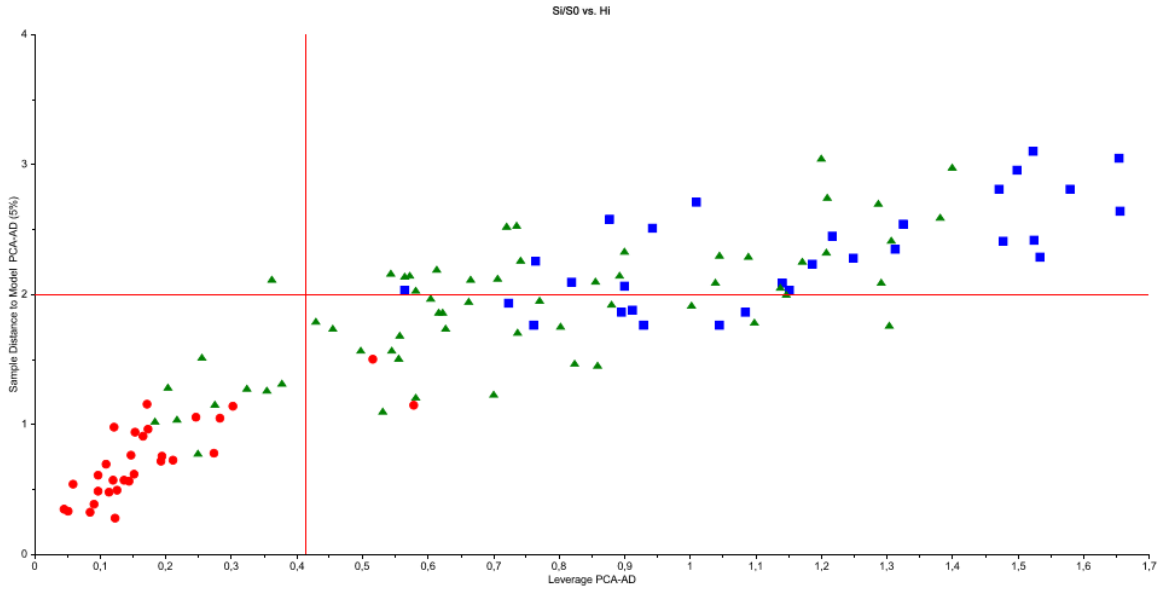
Figure 6. Discrimination power of the CERAD test scores in separating patients with Alzheimers's disease from healthy controls.



VFT24: Verbal Fluency test; CERADtot: total score; BNT: Boston Naming Test; WLM: Worl List Memory; CONPR: Constructional praxis; WLRecall: Word List Recall; WLrecogn: Word List Recognition;

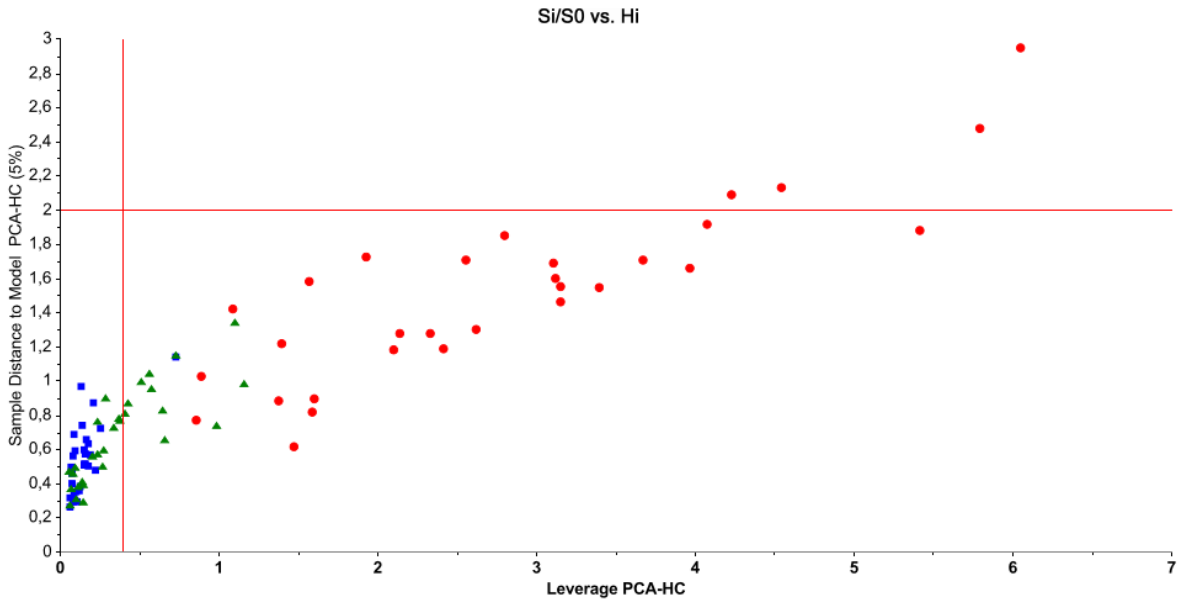
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

Figure 7. Si/S0 versus Hi plot with distances of the subjects in the calibration sets of patients with Alzheimer's disease (AD) (red dots) and healthy controls (HC) (blue squares) as well as patients with amnestic mild cognitive impairment (green triangles) to the AD principal component (PCA-AD) model.



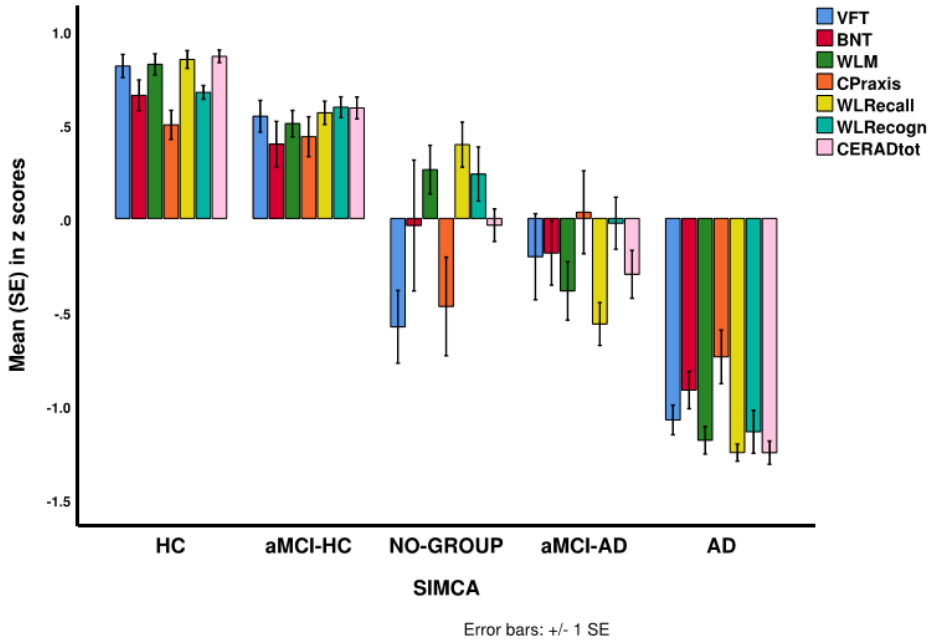
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

Figure 8. Si/S0 versus Hi plot with distances of the subjects in the calibration sets of patients with Alzheimer's disease (AD) (red dots) and healthy controls (HC) (blue squares) as well as patients with amnestic mild cognitive impairment (green triangles) to the HC principal component (PCA-HC) model.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

Figure 9. Mean (SE) values of the z scores of the CERAD tests and the total CERAD score (CERADtot) in patients with Alzheimer's Disease (AD) and healthy controls (HC) and amnestic mild cognitive impairment (aMCI), divided into three subgroups.



aMCI-HC: aMCI patients assigned by SIMCA to the HC class

aMCI-AD: aMCI patients assigned by SIMCA to the AD class

NO-GROUP: aMCI patients assigned by SIMCA neither to the AD nor the HC class

VFT: Verbal Fluency test; BNT: Boston Naming Test; WLM: Worl List Memory; CPraxis: Constructional praxis; WLRecall: Word List Recall; WLRecogn: Word List Recognition

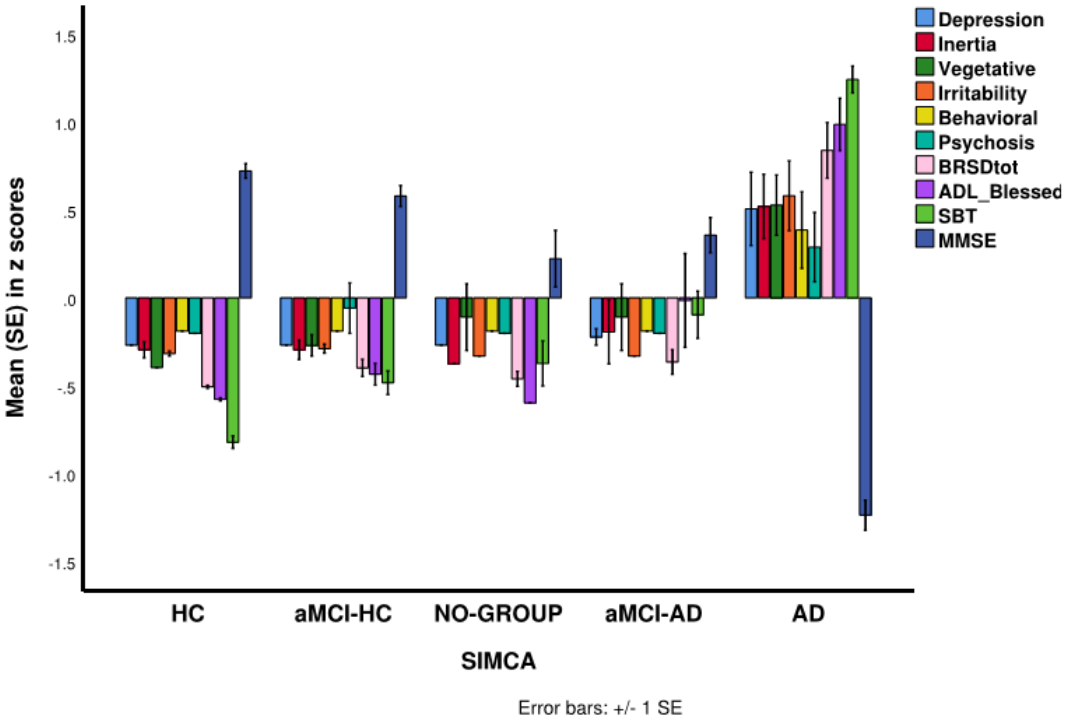
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

Figure 10. Mean (SE) values of the z scores of the CERAD tests and the total CERAD score (CERADtot) in patients with Alzheimer's Disease (AD) and healthy controls (HC) and amnestic mild cognitive impairment (aMCI), divided into three subgroups.

aMCI-HC: aMCI patients assigned by SIMCA to the HC class

aMCI-AD: aMCI patients assigned by SIMCA to the AD class

NO-GROUP: aMCI patients assigned by SIMCA neither to the AD nor the HC class



BRSD: Behavior Rating Scale for Dementia including depressive symptoms, inertia, vegetative symptoms, irritability/aggression, behavioral dysregulation and psychotic symptoms subscale scores; ADL_Blessed: CERAD Blessed Dementia Scale (BDS), to probe Activities of Daily Living (ADL); SBT: Short Blessed Test; MMSE: Mini-Mental State Examination.

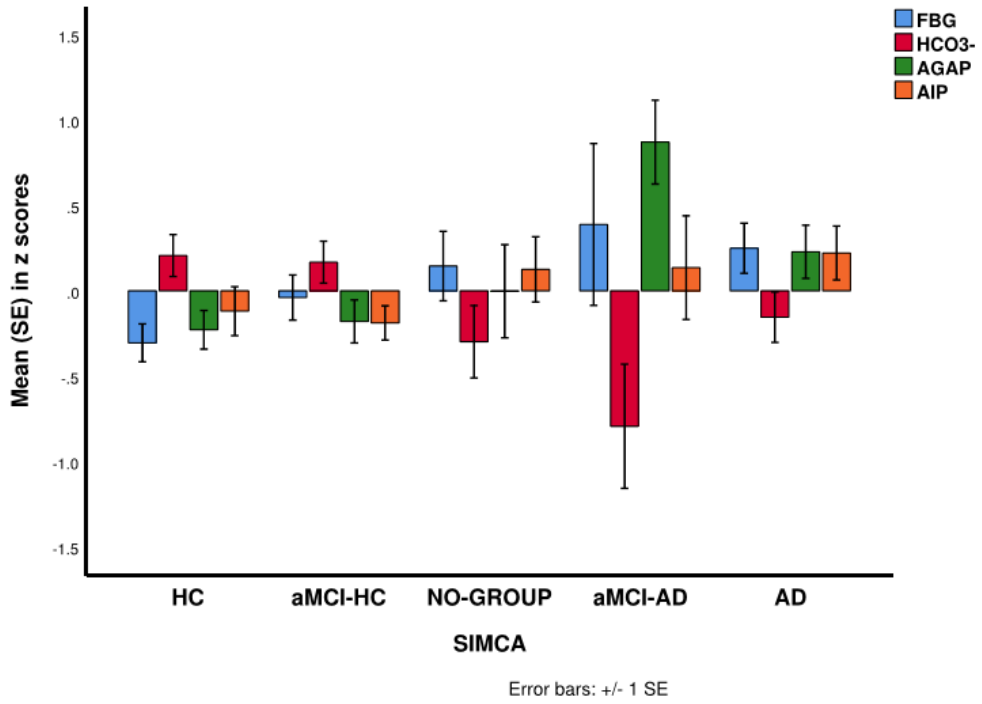
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

Figure 11. Mean (SE) values of the z scores of the CERAD tests and the total CERAD score (CERADtot) in patients with Alzheimer's Disease (AD) and healthy controls (HC) and amnestic mild cognitive impairment (aMCI), divided into three subgroups.

aMCI-HC: aMCI patients assigned by SIMCA to the HC class

aMCI-AD: aMCI patients assigned by SIMCA to the AD class

NO-GROUP: aMCI patients assigned by SIMCA neither to the AD nor the HC class



FBG: fasting blood glucose; HCO3-: bicarbonate: AGAP: anion gap; AIP: atherogenic index of plasma

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

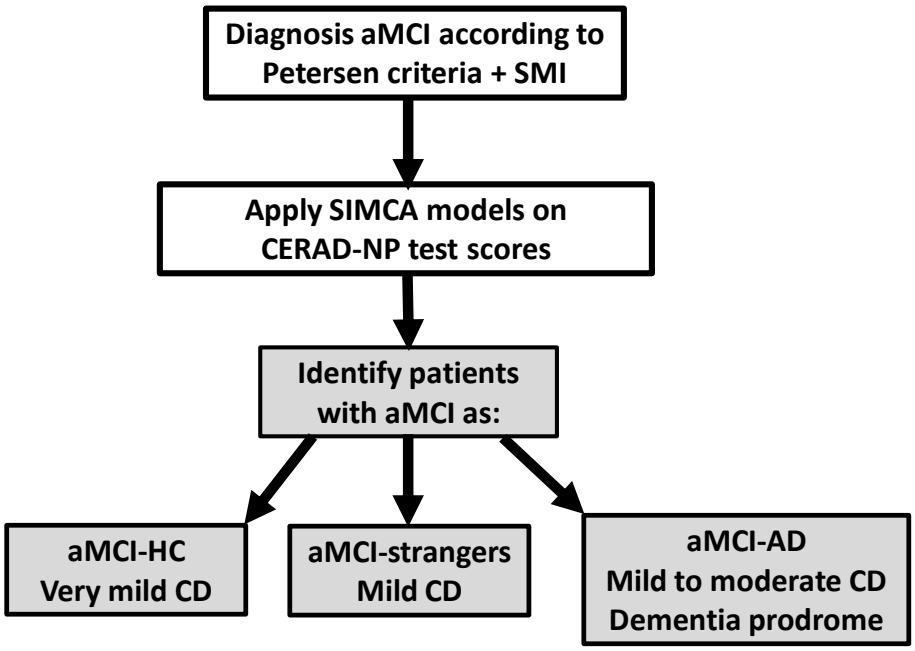
Figure 12. Indentification by SIMCA of patients with amnestic mild cognitive impairment into three different subgroups using the CERAD tests.

aMCI-HC: aMCI patients assigned by SIMCA to the HC class

aMCI-AD: aMCI patients assigned by SIMCA to the AD class

aMCI-strangers: aMCI patients assigned by SIMCA neither to the AD nor the HC class

CD: cognitive decline



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

Table 1. Demographic, clinical and biomarker data of healthy controls (HC), amnestic mild cognitive impairment (aMCI) and Alzheimer's disease (AD).

Variables	Total n=181			F/X^2	df	p
	HC(n=61)[A]	aMCI(n=60)[B]	AD(n=60)[C]			
Age	68.3 (5.5)[B,C]	74.8 (6.3)[A,C]	78.8 (7.1)[A,B]	42.94	2/178	0.000
Sex (M/F)	11/50	16/44	19/41	3.04	2	0.219
Education (years)	12.3 (5.0)[B,C]	10.0 (5.5)[A,C]	6.9 (5.7)[A,B]	15.17	2/178	0.000
CERAD Total	84.3 (5.9)[B,C]	72.8 (11.3)[A,C]	36.9 (10.8)[A,B]	398.46	2/178	0.000
VFT	21.1 (3.1)[B,C]	17.5 (4.6)[A,C]	9.2 (3.9)[A,B]	149.68	2/178	0.000
BNT	12.1 (2.0)[B,C]	10.8 (2.6)[A,C]	7.2 (2.4)[A,B]	70.93	2/178	0.000
WLM	22.8 (3.1)[B,C]	19.3 (3.8)[A,C]	8.6 (4.0)[A,B]	244.47	2/178	0.000
Constructional praxis	10.3 (1.4)[C]	9.7 (1.7)[C]	7.5 (2.5)[A,B]	34.94	2/178	0.000
WL True Recall	8.5 (1.3)[B,C]	6.8 (2.0)[A,C]	1.0 (1.3)[A,B]	385.10	2/178	0.000
WL Recognition	9.6 (0.9)[B,C]	8.8 (1.5)[A,C]	3.5 (3.0)[A,B]	164.99	2/178	0.000
Any ApoE4 (No/Yes)	52/9 [C]	52/8 [C]	26/34 [A,B]	19.09	2	0.000
FBG (mg/dL)	95.2 (17.9)[B,C]	102.7 (19.1)[A]	106.8 (23.6)[A]	5.00	2/177	0.008
HCO_3^- (mmol/L)	27.4 (2.8)	26.6 (2.6)	26.3 (3.3)	2.12	2/176	0.123
AGAP (mEq/L)	12.5 (2.4)[C]	13.1 (2.4)	13.7 (3.2)[A]	3.16	2/175	0.045
AIP (mg/dL)	2.11 (1.82)	2.17 (1.13)	2.69 (1.93)	1.98	2/168	0.127

[A,B,C] : pairwise comparisons between the three study samples

CERAD: The Consortium to Establish a Registry for Alzheimer's Disease

VFT: Verbal Fluency Test; BNT: Boston Naming Test; WLM: Word List Memory; FBG: Fasting blood glucose; HCO3-: Bicarbonate; AGAP: Anion GAP; AIP: Atherogenic index of plasma

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2019 doi:10.20944/preprints201905.0377.v1

Table 2. Demographic, clinical and biomarker data of patients with amnestic mild cognitive impairment (aMCI) divided into three subgroups according to SIMCA identification

Variables	Total n=60			F/X²*	df	P
	aMCI-HC (n=41)[A]	aMCI-strangers (n=10)[B]	aMCI-AD (n=9)[C]			
Age	73.9 (6.0)	75.5 (6.6)	78.0 (6.6)	22.46	4/176	0.000
Sex (M/F)	11/50	16/44	19/41	3.04	2	0.219
Education (years)	11.5 (5.2)[B]	5.8 (4.7)[A]	8.0 (5.0)	10.70	4/176	0.000
CERAD Total	78.1 (8.4)[B,C]	64.1 (6.2)[A]	58.2 (8.6)[A]	398.46	4/176	0.000
VFT	19.4 (3.4)[B,C]	12.3 (3.9)[A]	14.7 (4.4)[A]	100.29	4/176	0.000
BNT	11.2 (2.4)[C]	9.9 (3.4)	9.4 (1.6)[A]	37.84	4/176	0.000
WLM	20.5 (3.2)[C]	18.8 (3.0)[C]	14.2 (3.3)[A,B]	144.14	4/176	0.000
Constructional praxis	10.1 (1.5)[B]	8.1 (1.9)[A]	9.2 (1.5)	20.76	4/176	0.000
WL True Recall	7.5 (1.4)[C]	6.9 (1.4)[C]	3.4 (1.2)[A,B]	280.93	4/176	0.000
WL Recognition	9.3 (1.2)[C]	8.1 (1.5)	7.2 (1.4)[A]	88.56	4/176	0.000

*All results of analyses of variance or contingency analyses with Alzheimer's Disease patients, controls (see Table 1 for means) and the three aMCI subgroups as explanatory variable.

[A,B,C]: pairwise comparisons between the three study samples

CERAD: The Consortium to Establish a Registry for Alzheimer's Disease

VFT: Verbal Fluency Test; BNT: Boston Naming Test; WLM: Word List Memory