

Classification of Non-Demented Patients Attending a Memory Clinic using the New Diagnostic Criteria for Alzheimer's Disease with Disease-Related Biomarkers

Adrian Ivanoiu^{a,d,*}, Laurence Dricot^d, Nathalie Gilis^a, Cécile Grandin^{c,d}, Renaud Lhommel^b, Lisa Quenon^d and Bernard Hanseeuw^{a,d}

^aNeurology Department, Saint Luc University Hospital, Université catholique de Louvain, Brussels, Belgium

^bNuclear Medicine Department, Saint Luc University Hospital, Université catholique de Louvain, Brussels, Belgium

^cRadiology Department, Saint Luc University Hospital, Université catholique de Louvain, Brussels, Belgium

^dInstitute of Neuroscience, Université catholique de Louvain, Brussels, Belgium

Handling Associate Editor: Sebastiaan Engelborghs

Accepted 2 July 2014

Abstract.

Background: New diagnostic criteria for predemential Alzheimer's disease (AD) advocate the use of biomarkers. However, the benefit of using biomarkers has not been clearly demonstrated in clinical practice.

Objective: To investigate whether a combination of biomarkers may be helpful in classifying a population of non-demented patients attending a Memory Clinic.

Methods: Sixty non-demented patients were compared with 31 healthy elderly subjects. All subjects underwent a neuropsychological examination, brain 3T magnetic resonance imaging, [F18]-fluorodeoxyglucose and [F18]-flutemetamol positron emission tomography. According to their performance on memory, language, executive, and visuo-spatial domains, the patients were classified as mild cognitive impairment (amnesic, non-amnesic, single, or multiple domain) or subjective cognitive impairment. Patients were then classified according to the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria, using the normalized mean hippocampal volume (Freesurfer), [F18]-FDG PALZAD, and [F18]-flutemetamol standard uptake value ratio (SUVR) (cut-off at the 10th percentile of controls). The standard of truth was the clinical status at study entry (patient versus control).

Results: The sensitivity/specificity of the clinical classification was 65/84%. The NIA-AA criteria were applicable in 85% of patients and 87% of controls. For biomarkers the best sensitivity (72%) at a fixed specificity of 84% was achieved by a combination of the three biomarkers. The clinical diagnosis was reconsidered in more than one third of the patients (42%) as a result of including the biomarker results.

Conclusions: Application of the new NIA-AA AD diagnostic criteria based on biomarkers in an unselected sample of non-demented patients attending a Memory Clinic was useful in allowing for a better classification of the subjects.

Keywords: Alzheimer's disease, biological markers, early diagnosis, mild cognitive impairment

INTRODUCTION

Non-demented patients attending a memory and cognitive disorders clinic are classified as having mild cognitive impairment (MCI) if their cognitive

*Correspondence to: Adrian Ivanoiu, MD, PhD, Saint Luc University Hospital/Neurology Department, Av. Hippocrate, 10, Brussels 1200, Belgium. Tel.: +32 2 764 21 46; Fax: +32 2 764 90 46; E-mail: adrian.ivanoiu@uclouvain.be.

performance is considered as impaired or subjective cognitive impairment (SCI) otherwise [1, 2]. Most MCI patients have amnesic MCI (aMCI) because their memory deficit is predominant, but non-amnesic presentations of MCI (naMCI) also exist. Single and multiple domain deficit subtypes have been defined for both aMCI and naMCI [3]. A considerable bulk of research has shown that MCI patients are at a greater risk of developing dementia and, particularly, Alzheimer's disease (AD) than are control subjects of the same age (for a review, see [4]). It is, however, acknowledged that MCI is a heterogeneous condition, with some patients evolving to dementia and others remaining stable or even recovering normal cognition [5–7]. aMCI patients are more predisposed to develop AD than are naMCI patients and those with multiple domain deficits are probably at a later stage of the disease and likely to progress more rapidly [3, 4]. In turn, SCI patients less frequently develop AD, but their risk of cognitive deterioration is still greater than that of healthy elderly control subjects [2, 8]. It is, however, very difficult for the clinician to distinguish with sufficient accuracy those patients at high risk of developing AD solely on the basis of clinical and cognitive examinations.

Recently, a National Institute on Aging-Alzheimer's Association (NIA-AA) group of experts issued new diagnostic criteria for AD, including its predementia stages [9]. The authors claimed that the early diagnosis of AD could be improved by using biological markers (biomarkers) related to the disease process and evolution [10]. One class of biomarkers is believed to reflect an abnormally high deposition of the amyloid- β peptide ($A\beta$) in the brain and can be assessed with appropriate amyloid positron emission tomography (PET) tracers [11]. A second class of AD biomarkers represents the early modifications related to the neurodegeneration process (particularly neuronal and synaptic damage) resulting in cortical metabolism decline and brain atrophy [12]. Previous research on biomarkers has consistently shown that most MCI patients who subsequently develop AD have abnormal biomarkers [13–16], but a significant number of elderly patients without cognitive impairment also have abnormal biomarker values [17, 18]. Although many of these biomarkers are now available in clinical practice, methods lack standardization and validation, particularly in unselected clinical populations [19]. Moreover, it has not been clearly established which combination of biomarkers offers the best sensitivity/specificity balance. This issue is of considerable importance if biomarker assessment is to be incorpo-

rated into routine clinical use as suggested by the new AD diagnostic criteria [9, 10].

In the present study, we investigated whether a combination of biomarkers as suggested in the NIA-AA recommendations was useful in improving the classification of a typical clinical population of non-demented patients attending a Memory Clinic. More specifically, we aimed to evaluate the contribution of biomarkers beyond the clinical and neuropsychological assessment.

MATERIALS AND METHODS

Subjects

The study was conducted at the Memory Clinic of Saint Luc University Hospital, Brussels and was approved by the local Ethical Committee (Eudra-CT number: 2011-001756-12). All subjects provided informed consent. Sixty-seven consecutive non-demented patients attending the Clinic between January 2012 and April 2013 were evaluated for possible inclusion. All patients were assessed clinically by one of two experienced investigators (AI and BH). Inclusion criteria included age at least 50 years old and complaints of memory and/or other cognitive problems. Evidence from a relative was sought and obtained for the majority of the patients. Dementia was excluded using DSM-IV-TR criteria [20] and a Mini-Mental State Exam (MMSE) score of 24 or more was required [21]. Exclusion criteria also comprised major depression or other psychiatric diseases, past or present neurological conditions, and alcohol and drug abuse. Of the patients who attended the Clinic during the specified period, 60 completed the study: five patients did not complete the whole assessment, one patient was excluded for having undergone cranial surgery, and another was reclassified as demented after having obtained more information on his everyday life difficulties. The patients were compared with a control group composed of 32 healthy elderly recruited by advertisements. Most had been doing volunteer work at the hospital, while others heard of the study from family or friends. None of these subjects complained of their cognition and memory beyond what is expected from persons of their age. One control subject was excluded because of the presence of a systemic illness potentially leading to cognitive impairment.

All subjects underwent the same assessment consisting of a comprehensive neuropsychological examination, an evaluation of biomarkers (see *Neuropsychological assessment* and *Biomarkers assessment*

sections) and apolipoprotein E genotyping. The microvascular load was evaluated by a trained neuro-radiologist (CG) from 4 mm thick axial FLAIR (FLuid Attenuated Inversion Recovery) magnetic resonance (MR) images using the Manolio scale [22].

Neuropsychological assessment

The neuropsychological assessment evaluated episodic memory, language, executive functions, and visuo-spatial processing. Episodic memory was tested using the Free and Cued Selective Reminding Test, which requires memorization of 16 visually presented words belonging to 16 different semantic categories used as cues in order to control encoding and retrieval (French version, see [23]). Language was tested using the LEXIS Naming Test [24], the Category Fluency Test for animals, and the Letter Fluency Test for the letter P [24]. Executive functions were evaluated using the Trail Making Test and Luria's Graphic Sequences (adaptations in French, unpublished). Visuo-spatial processing was examined using the Clock Drawing Test [25] and the Praxis part of the CERAD battery [26].

Biomarker assessment

The biomarker assessment included volumetric brain MRI, a fluorine-18 fluoro-2-deoxyglucose ([18F] FDG) and [F18]-flutemetamol PET scan as explained below (a more detailed description of the procedure can be found in the Supplementary Material).

- a) *Volumetric brain MRI*: For each subject, 3D heavily T1-weighted images were recorded at 3T (Achieva, Philips Healthcare) with a 32-channel phased array head coil. The 3D sequence consisted of a gradient echo sequence with an inversion prepulse (Turbo Field Echo) acquired in the sagittal plane using the following parameters: TR/TE/flip angle = 9.1 ms/4.6 ms/8°, 150 slices, slice thickness = 1 mm, in-plane resolution = $0.81 \times 0.95 \text{ mm}^2$ (acquisition) reconstructed in $0.75 \times 0.75 \text{ mm}^2$, FOV = $220 \times 197 \text{ mm}^2$, acquisition matrix = 296×247 (reconstruction 3202), SENSE factor = 1.5 (parallel imaging). Whole brain was segmented by completing the FreeSurfer image analysis pipeline [27]. The technical details of these procedures have been described in prior publications [28–30]. Subsequently, we computed the mean (from left and right) hippocampal

volume normalized to the intracranial volume according to a regression equation obtained from the whole group of participants. The Normalized Hippocampal Volume (N_HVOL) was calculated via the equation: $y' - y$ where y' is the measured hippocampal volume and y is the theoretical value of the hippocampal volume according to the Intracranial Volume (ICV). This later term was defined as equating:

- for the left hippocampus, $y = 3427,0646 + 0,00009987 x$
- for the right hippocampus, $y = 3415,1737 + 0,00007724 x$

where x is the ICV of each participant. The resulting values can be negative when the measured volume is smaller than the expected volume according to the ICV.

- b) *[F18]-flutemetamol PET*: [F18]-flutemetamol is an investigational medicinal product being studied clinically as an amyloid imaging agent. The study was conducted according to ICH-GCP recommendations after receiving authorization from the national legally competent authorities (AFMPS-FAGG). [F18]-flutemetamol was produced and batch manufactured according to Good Manufacturer Practice Guidelines in Belgium (Centre de Recherche du Cyclotron, Sart-Tilman, ULg) as described previously [31] and supplied as a ready to use injectable solution. After intravenous injection of a target activity of 175–185 MBq of [F18]-flutemetamol, a 30-min list mode PET/CT acquisition was performed on a Philips Gemini TF 16 slices (Philips Healthcare) 90 min post-injection and reconstructed as a dynamic scan of $6 \times 5 \text{ min}$, with 2 mm isometric voxels including attenuation, scatter, decay corrections and time-of-flight information using the standard manufacturer's TF reconstruction algorithm (3 iterations $\times$ 8 subsets). Visual qualitative analysis (following criteria defined in a dedicated training program provided by GE Healthcare) and quantitative analysis were performed. Quantitative standard uptake value ratios (SUVr) were computed using the region of interest defined in the MNI space and adapted to the patient's anatomy after applying inverse transformation into the patient space using PVIEW and PFUS v3.2.2 software modules [32].
- c) *[F18]-FDG PET*: All patients underwent a 7-min list mode [18F] FDG PET/CT study according to

published guidelines [33] after intravenous injection of target activity of 146–185 MBq of [18F] FDG and reconstructed by the standard manufacturer's TF reconstruction algorithm. [18F] FDG PET studies were then post-processed using PMOD Alzheimer's discrimination analysis with PALZ v2.75 software [32], previously validated in a longitudinal study of a MCI patient population [34]. ADt-sum scores were converted to PALZAD scores on an Excel sheet using the following equation: $\text{PALZAD score} = \log_2[(\text{ADt-sum}/11.089) + 1]$ [35].

Classification

- a) Clinical classification: From the clinical and neuropsychological examination, subjects could be rated as cognitively impaired or not impaired. For each of the four cognitive domains (memory, language, executive, and visuospatial), three measures were taken into account. If a patient had a performance below the 10th percentile of an independent historical control group on two of the three measures in at least one cognitive domain he/she was classified as having MCI. This method was chosen in order to minimize random variability of performance. The independent control group, originating from our database, is currently used to classify the patients that underwent a neuropsychological evaluation and it is stratified according to gender, age, and education. According to the cognitive domain involved and the presence of one or multiple impairments, MCI patients were classified as aMCI or naMCI, and single domain or multiple domain. Patients with a performance above the threshold on all four domains were considered as having SCI.
- b) Classification using biomarkers and NIA-AA criteria: For each biomarker, one measure was considered: the Normalized Hippocampal Volume; the [F18]-FDG PALZAD score; and the [F18]-flutemetamol SUVR. For the sake of simplicity, these three measures are labeled "N_HVOL", "PET-FDG", and "PET-amyloid", respectively. The results were considered as impaired if they scored below the 10th percentile of the control subjects group of this study. MCI patients were ranked in accordance with Albert et al. [10]. Controls and SCI subjects were rated according to Jack et al. [36] as stage 0 if all the markers were negative, stage 1 if only the PET-amyloid was positive, stage 2 if at

least one neurodegeneration marker (PET-FDG or N_HVOL) was positive in addition to a positive PET-amyloid, and suspected non-AD pathology (SNAP) if at least one neurodegeneration marker (PET-FDG or N_HVOL) was positive but the PET-amyloid was negative. As, by definition, cognition in the SCI group was considered as non-impaired we did not use in this group the stage 3 (similar to stage 2 but in addition some subtle cognitive impairment detected).

Statistics

Group comparisons (patients versus controls) for the demographic characteristics and the neuropsychological examination were performed. A one-way analysis of variance was used for age, microvascular disease, and the MMSE. Categorical data were assessed with frequency tables and the Chi-squared Test. As the assumption of normality was rejected in most cases by Levene's Test for Equality of Variances, a nonparametric test (Mann-Whitney) was used for continuous variables from the cognitive and biomarker evaluation. The three biomarkers were also compared with ROC curves. For determining sensitivity/specificity the standard of truth was the clinical comparator (patients versus controls) for both the cognitive and biomarker measures. Therefore, the sensitivity is represented by the % of patients with impaired cognition or positive markers respectively whereas specificity is the % of controls with normal cognition or normal markers. Different combinations of biomarkers were also assessed by computing individual z-scores for each marker and then calculating composite scores corresponding to the average of each possible combination of two and three markers. These composite scores were also converted to z-scores. For all statistical analyses, the MedCalc software was used [37].

RESULTS

Subjects' characteristics and neuropsychological examination

The subjects' characteristics are shown in Table 1.

There were no statistically significant differences between patients and controls for age, gender, or educational level. The degree of microvascular charge was small and similar in patients and controls. Although a higher percentage of patients (53%) showed presence of the apolipoprotein E (ApoE) $\epsilon 4$ allele compared to controls (32%), the difference between groups did not

Table 1
Participants' characteristics

	Controls <i>n</i> = 31	Patients <i>n</i> = 60	<i>p</i> -value
Age (y)	70.0 (5.5)	70.8 (8.0)	0.5910
Gender (% male)	48%	55%	0.7059
Education (level 1/2/3%)	6/13/81%	17/23/60%	0.2159
ApoE genotyping (% ApoE ε4)	32%	53%	0.1104
Microvascular disease (Manolio 0–9)	2.3 (1.3)	2.6 (1.7)	0.4230
MMSE (0–30)	29.1 (1.1)	26.8 (1.8)	<0.0010

Values are mean (SD); Education level: 1=primary school, 2=secondary school, 3=more than secondary school. Manolio: scale describing the degree of microvascular lesions (from 0=absence of lesions to 9=the highest degree); MMSE: Mini-Mental State Exam.

reach the threshold of statistical significance. Patients had a significantly lower score on the MMSE compared to controls.

The subjects' cognitive performances are presented in Table 2 (participants' z-scores relative to the matched controls from the independent control group are available as supplementary material, see Supplementary Table 1).

There was a statistically significant difference between patients and controls for all measures except the Luria Graphical Test and the Clock Drawing Test.

Clinical classification according to the cognitive performance and pattern of deficits

The classification of the patients and the control group is shown in Fig. 1.

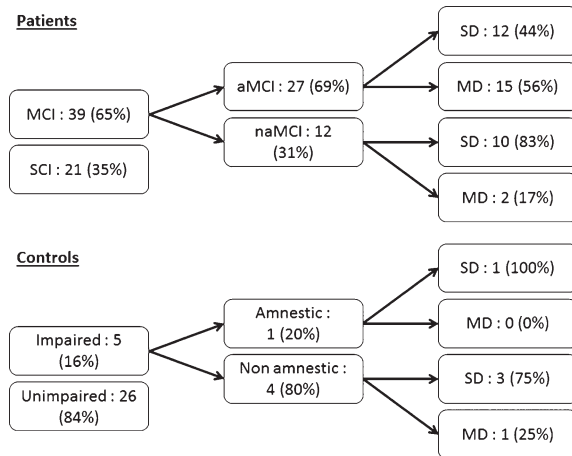


Fig. 1. Clinical classification. Values are raw numbers (%); SCI, Subjective Cognitive Impairment; MCI, Mild Cognitive Impairment; aMCI, amnesic MCI; naMCI, non amnesic MCI; SD, single domain; MD, multiple domain.

Within the control group, 5 subjects were found to be cognitively impaired: four with single domain (1 for memory and 3 for executive functions) and one multiple domain (for executive and visuo-spatial functioning). Therefore, the clinical classification including the neuropsychological evaluation led to a sensitivity/specificity of 65/84%.

The biomarker assessment and discriminating power

The results of the three biomarkers (N_HVOL, PET-FDG, PET-amyloid) assessed in controls, patients and

Table 2
Participants' cognitive performances

Test		Controls		Patients		<i>p</i> -value
	min/max	<i>n</i> = 31		<i>n</i> = 60		
<i>Memory</i>						
RL/RI 16 – RL sum of trials	0–48	32.0	28.0–36.5	22.5	15.0–28.3	<0.0001
RL/RI 16 – RT sum of trials	0–48	47.0	45.5–48.0	43.0	34.5–46.0	<0.0001
RL/RI 16 – RL delayed	0–16	13.0	11.0–14.0	9.0	4.5–12.0	<0.0001
<i>Language</i>						
Naming Test	0–64	61.0	58.0–62.0	55.0	52.0–58.3	<0.0001
Category Fluency	0–	35.0	29.5–39.5	26.0	21.0–33.3	<0.0001
Phonological Fluency	0–	22.0	19.0–30.0	17.5	12.0–23.0	0.0001
<i>Executive</i>						
Luria Graphical Test	0–32	28.0	24.8–30.0	24.8	22.6–29.5	0.0980
TMT B-A time	sec	50.5	33.8–71.5	74.5	53.0–139.5	0.0012
TMT B-A errors	0–20	0.0	0.0–0.0	1.0	0.0–1.0	0.0171
<i>Visuo-spatial</i>						
CERAD 4 figures	0–11	11.0	10.0–11.0	10.0	10.0–11.0	0.0019
Clock drawing	0–8	8.0	6.0–8.0	7.0	6.0–8.0	0.2117
Clock copy	0–10	10.0	10.0–10.0	10.0	9.0–10.0	0.0445

Values are median, quartile 25–75; RL/RI 16: Rappel libre/Rappel indicé 16; RL: Rappel Libre; RT: Rappel libre + indicé; TMT: Trail Making Test; Statistics were performed using the Mann-Whitney Test.

Table 3
The biomarker assessment

A. In controls versus patients									
	Controls (<i>n</i> = 31)		Patients (<i>n</i> = 60)			C/P	C/SCI	C/MCI	SCI/MCI
		All (<i>n</i> = 60)	SCI (<i>n</i> = 21)	MCI (<i>n</i> = 39)					
PET amyloid	1.33 (1.27–1.46)	1.52 (1.31–2.03)	1.32 (1.27–1.45)	1.86 (1.41–2.07)		0.0022	0.8961	<0.0001	0.0002
PET FDG	0.45 (0.20–0.61)	0.86 (0.63–1.47)	0.63 (0.40–0.83)	0.98 (0.73–1.61)		<0.0001	0.0481	<0.0001	0.0003
N_HVOL	236.3 (49.9–476.3)	−172.7 (−506.0– −333.9)	71.6 (−203.9– −464.3)	−359.5 (−550.7– −32.7)		0.0016	0.2514	0.0002	0.0261
B. In controls versus amnesic and non amnesic MCI									
	Controls (<i>n</i> = 31)	aMCI (<i>n</i> = 27)	naMCI (<i>n</i> = 12)		C/aMCI	C/naMCI	aMCI/naMCI		
PET amyloid	1.33 (1.27–1.46)	1.86 (1.48–2.08)	1.88 (1.30–2.04)		<0.0001	0.0580	0.4377		
PET FDG	0.45 (0.20–0.61)	1.03 (0.81–1.52)	0.81 (0.71–1.93)		<0.0001	0.0003	0.5839		
N_HVOL	236.3 (49.9–476.3)	−387.4 (−701.5– −38.83)	−193.62 (−493.6– −615.8)		0.0001	0.1227	0.2012		
C. In controls versus single domain and multiple domain MCI									
	Controls (<i>n</i> = 31)	aMCI–SD (<i>n</i> = 12)	aMCI–MD (<i>n</i> = 15)	naMCI–SD (<i>n</i> = 10)	naMCI–MD (<i>n</i> = 2)	C/aMCI - SD	C/aMCI - MD	C/naMCI - SD	aMCI– SD/MD
PET amyloid	1.33 (1.27–1.46)	1.75 (1.41–2.06)	2.02 (1.54–2.08)	1.68 (1.24–2.02)	2.25	0.0018	<0.0001	0.2132	0.3930
PET FDG	0.45 (0.20–0.61)	0.96 (0.72–1.38)	1.39 (0.90–1.52)	0.80 (0.69–1.72)	1.57	0.0001	<0.0001	0.0010	0.3413
N_HVOL	236.3 (49.9–476.3)	−113.0 (−465.7– −506.3)	−499.3 (−846.6– −366.5)	−69.1 (−448.3– −625.2)	−573.5	0.0986	<0.0001	0.3786	0.0359

Values are median (quartile 25–75) and represent the [F18]-flutemetamol SUVR score for PET amyloid, the [F18]-FDG PALZAD score for PET FDG and the mean hippocampal volume normalized to the intracranial volume (N_HVOL); Statistics were performed using the Mann-Whitney Test (*p*-value shown); C: healthy controls; SCI: subjective cognitive impairment; MCI: mild cognitive impairment; aMCI: amnesic MCI; naMCI: non amnesic MCI; SD: single domain; MD: multiple domain; only pertinent comparisons are shown.

patient subgroups are shown in Table 3A–C (the raw values for the mean hippocampal volume are available as supplementary material, see Supplementary Table 2A–C).

The three biomarkers could distinguish between patients and controls (Table 3A). The SCI group was indistinguishable from controls but significantly different from the MCI patients for the PET-amyloid and the N_HVOL. In contrast, SCI patients were hypometabolic according to the PET-FDG compared to controls, and still different from the MCI patients. All three biomarkers were significantly impaired in the MCI group compared to the controls. When the classification into amnesic and non-amnesic MCI was taken into account (Table 3B), the aMCI were impaired for all three biomarkers when compared to controls whereas the naMCI showed intermediate results between controls and aMCI. They were not significantly different from the aMCI and when compared to controls only the PET-FDG reached statistical significance (a strong trend was observed for the PET-amyloid). Within the MCI group (Table 3C), the naMCI multiple domain group (two patients) was not considered for the analysis. Both aMCI single domain and multiple domain were different from controls for PET-amyloid and PET-FDG. However, only the aMCI multiple domain

were atrophic, whereas only a trend was observed for aMCI single domain. There were no significant differences in PET-amyloid or PET-FDG between these two subgroups, but patients with aMCI multiple domain tended to have more hippocampal atrophy than did their single domain counterparts. naMCI single domain patients were not different from controls for the PET-amyloid and N_HVOL but they were significantly more hypometabolic on PET-FDG.

Pairwise comparisons with ROC curves revealed that the three biomarkers were not different in their capacity to distinguish between controls and patients (see Table 4).

The combinations of two or three biomarkers that included the PET FDG discriminated significantly better between patients and controls than each biomarker individually. At a fixed cut-off resulting in a specificity similar to the one obtained for the clinical classification (84%), the best sensitivity score (72%) was obtained by a combination of the three biomarkers.

Classification according to the NIA-AA criteria

For the binary classifications we used the following cut-offs for the three biomarkers: >1.622 for

Table 4
Pairwise comparisons with ROC curves

	ROC AUC (SE)	Sensitivity (at 84% specificity)	Comparisons ROC significant at $p < 0.05$
(1) PET Amyloid	0.697 (0.0556)	48	(5); (7)
(2) N_HVOL	0.703 (0.0552)	50	(6); (7)
(3) PET FDG	0.808 (0.0484)	42	–
(4) PET Amyloid + N_HVOL	0.727 (0.0524)	62	(5); (6); (7)
(5) PET Amyloid + PET FDG	0.798 (0.0476)	62	(1) 0.0053 (4) 0.0244
(6) N_HVOL + PET FDG	0.775 (0.0474)	63	(2) 0.0148 (4) 0.0373
(7) PET Amyloid + N_HVOL + PET FDG	0.779 (0.0487)	72	(1) 0.0436 (2) 0.0374 (4) 0.0040

AUC(SE), Area under the curve (standard error); analysis included individual biomarkers (PET amyloid, N_HVOL, and PET FDG) and their combinations computed as composite scores (mean of z-scores of individual markers) transformed themselves in z-scores. p values are shown only once for each comparison. The sensitivity score corresponding to a specificity set at 84% (similar to the one of the clinical classification) is displayed.

PET-amyloid; >0.994 for the PET-FDG, and <-253.63 for N_HVOL (corresponding to the 10th percentile of the control group).

The resulting classification for controls and patients is shown in Table 5.

Within the control group, the unclassified subgroup was composed of four subjects with cognitive impairment but negative biomarkers. The SCI subgroup included a percentage of negative biomarker individuals comparable to that in the control group. However, SCI patients with positive biomarkers were different from their control counterparts because they often cumulated two or three positive markers. Among the six SCI patients in this situation, five were positive for at least two biomarkers, whereas among the eight respective controls only one had two positive biomarkers. As expected, a majority of MCI patients (56%) were classified as “due to AD” with high likelihood according to the NIA-AA criteria as they have both a positive marker for amyloid deposition and at least one of the markers of neurodegeneration positive. A strict application of the NIA-AA criteria resulted in 8 (21%) of the MCI patients being “unlikely due to AD” (all three biomarkers negative) and 9 patients (23%) “MCI-core” because the results could be considered as conflicting (either a positive PET-amyloid with negative PET FDG and N_HVOL or a negative PET-amyloid with at least one neurodegeneration marker positive). However, if the same logic of classification used for unimpaired participants (controls and SCI) is applied to the MCI then 11 (28%) of them became “unclassifiable” (8 patients with all biomarkers negative and 3 with a positive PET-amyloid but negative biomarkers of neurodegeneration) whereas 6 (15%) patients with at least one marker of neurodegeneration positive but negative PET-amyloid are considered as the equivalent of SNAP. The groups “due to AD” and “core” were comparable in terms of the percent-

age of aMCI (73% and 78% respectively) and multiple domain MCI (55% and 56%). Of notice, among the group “unlikely due to AD” all patients were single domain (50% aMCI and 50% naMCI). Therefore, the method using three biomarkers and the NIA-AA criteria classified 27 out of 31 controls (87%) and 51 out of 60 patients (85%). Alternatively, when the same classification used for unimpaired participants (controls and SCI) is applied to the MCI 49 out of 60 patients were classified (82%).

Classification using biomarkers versus clinical diagnosis

When the method using biomarkers and the NIA-AA classification was applied to the sample that was already classified on basis of the neuropsychological examination, the clinical diagnosis was either confirmed or challenged. We considered that changing the diagnostic label was required in four situations: 1) when the patient was rated as cognitively normal (SCI) but biomarkers were positive (at least one of them); 2) when the patient was rated MCI (amnesic or non-amnesic) but biomarkers were negative (all of them); 3) when the patient was diagnosed as aMCI, therefore suspected of AD but biomarkers showed a rather non-AD amyloid negative pattern; 4) when the patient was diagnosed as naMCI (presumably non-AD) but the biomarkers showed he/she was amyloid positive. In summary, these cases diagnosed according to the neuropsychological pattern proved to be false negative, false positive, false AD-type, or false non AD-type cognitive pattern after the biomarkers. For this classification, we considered that MCI patients with normal biomarkers were, in fact, false positives and only the MCI patients who were amyloid positive and neurodegeneration negative could not be classified. This reclassification is shown in Fig. 2.

Table 5
Classification according to the NIA-AA criteria

		PET amyloid	PET FDG	N_HVOL	Cognition	%	Overall %
Controls	stage 0	—	—	—	—	61	61
	stage 1	+	—	—	—	7	7
	stage 2	+	+	—	—	3	3
		+	—	+	—	0	
		+	+	+	—	0	
		+	+	—	+	0	
	stage 3	+	+	+	+	0	0
		+	—	+	+	0	
	SNAP	+	+	+	+	0	
		—	+	—	—	6	16
		—	+	—	+	0	
		—	—	+	—	7	
		—	—	+	+	3	
	unclassified	—	+	+	—	0	
		—	+	+	+	0	
		—	—	—	+	13	13
		+	—	—	+	0	
SCI	stage 0	—	—	—	—	71	71
	stage 1	+	—	—	—	5	5
	stage 2	+	+	—	—	0	10
		+	—	+	—	5	
	SNAP	+	+	+	—	5	
		—	+	—	—	4	14
		—	—	+	—	0	
		—	+	+	—	10	
MCI	due to AD	+	+	—	+	5	56
		+	—	+	+	18	
		+	+	+	+	33	
	“core”	+	—	—	+	8	23
		—	+	—	+	10	
		—	—	+	+	5	
		—	+	+	+	0	
	unlikely AD	—	—	—	+	21	21

Values are %; ±: impaired/unimpaired at cut-off 10th percentiles of controls; N_HVOL: normalized hippocampal volume; SCI: Subjective Cognitive Impairment; MCI: Mild Cognitive Impairment; SNAP: suspect of non-AD pathology; AD: Alzheimer's disease; “core”: core MCI.

We can observe that the application of the biomarker method after the method based on the neuropsychological pattern led to a reclassification of 25 out of the 60 patients (42%).

DISCUSSION

In the present study, we tried to investigate the value of biomarkers in everyday clinical practice. We, therefore, included all consecutive non-demented patients who attended our Memory Clinic and examined them as the physician normally would proceed by first making a clinical diagnosis (including the neuropsychological examination) and then using a comprehensive biomarker assessment and examining its impact on the final diagnosis. The key findings of our study were: first, a clinical classification distinguished cognitively impaired from unimpaired subjects in a sample of patients and supposedly healthy controls

with a sensitivity/specificity of 65/84%; second, when the three biomarkers (PET-amyloid, PET-FDG, and N_HVOL) were taken into consideration separately, they were neither different from one another in discriminating cases from controls nor superior to the clinical and neuropsychological method; third, combined biomarkers were superior to both the individual biomarkers and to the clinical method, especially when the three markers composite score is considered (sensitivity/specificity: 72/84%); fourth, the NIA-AA criteria of classification were applicable in 51 patients out of 60 (85%) and the use of biomarkers led to a reconsideration of the clinical diagnosis in more than one third of the patients (42%). These different aspects will be discussed below.

Patients who complain about memory function are traditionally classified according to their performance on cognitive tests. We used such a classification and found that 65% of individuals presenting to our memory clinic could be considered as having MCI. We used

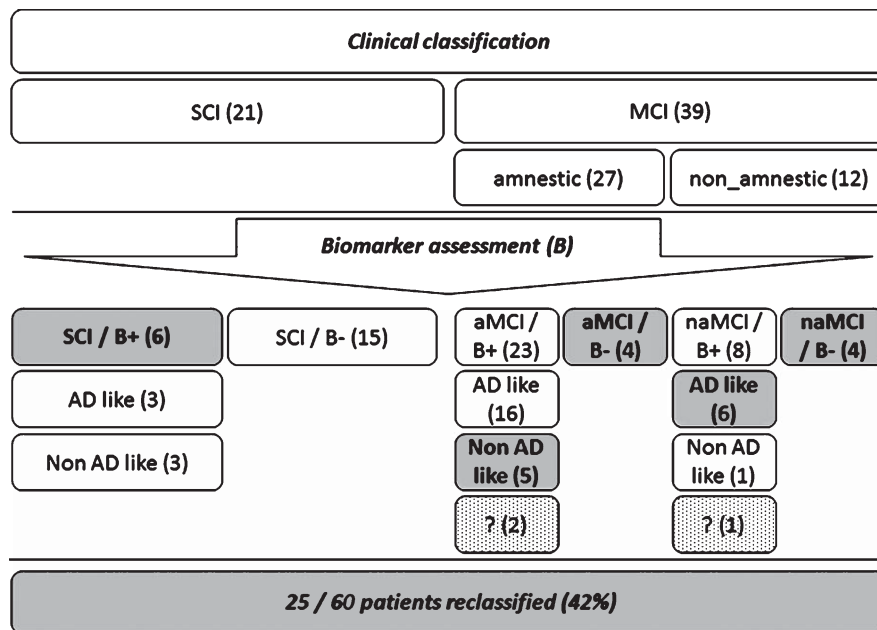


Fig. 2. Classification using biomarkers versus clinical diagnosis. Values are (raw numbers); SCI: Subjective Cognitive Impairment; MCI: Mild Cognitive Impairment; aMCI: amnestic MCI; naMCI: non amnestic MCI; B+: biomarker (any of the PET-amyloid, PET-FDG, or N_HVOL) positive (cut off at 10th percentile of controls); B-, all three biomarkers negative; patients marked with bold font and included in dark gray squares needed to be reclassified when the biomarkers status was taken into account; ? unclassifiable MCI patients amyloid positive and neurodegeneration negative; AD like: suspected Alzheimer's disease with positive PET-amyloid irrespective of PET-FDG or N_HVOL; Non AD like: suspected non-Alzheimer dementia with negative PET-amyloid but positive PET-FDG or N_HVOL.

a comprehensive neuropsychological battery, including tests widely employed in this setting, so we are confident that the evaluation reflects usual practice. For the remaining 35% of patients, the neuropsychological examination was non-informative, showing no deficit despite complaints of poor memory, so these patients were classified as having SCI. However, the accuracy of the neuropsychological classification may be limited in patients who have only small deficits, difficult to distinguish from the effect of aging. This shortcoming could result in false negative results, i.e., individuals with apparently normal cognition but who will nevertheless evolve to dementia, but also in to false positive results, i.e., individuals who appear to have impaired cognition (MCI) but do not later progress to dementia. In both cases, the information given to the patients and their families will not be correct, and this can have unfortunate consequences if the patient believes they have incipient dementia when this is not the case or, in contrast, if they think they are in good health whereas, in fact, deterioration will soon follow.

To help the clinician to resolve this problem and to enable more accurate predictions, the NIA-AA experts favor the use of AD-related biomarkers. In this study, we selected PET-FDG and N_HVOL, in agreement

with the NIA-AA guidelines and with other recent studies [36]. To measure brain metabolism with PET-FDG, we used the method developed by Herholz et al. [38], because it is believed to reflect the uptake of brain regions more closely related to the development of AD. Hippocampal atrophy is one of the earliest neurodegeneration signs in AD, as shown by many studies (see [39] for a review). We measured the N_HVOL using an automated method from Freesurfer and corrected this measure for the intracranial volume as has already been done in this setting [36]. The presence of abnormal amyloid deposits in the brain, as reflected by a high uptake of amyloid specific tracers in PET, is the hallmark of AD in both preclinical and MCI stages [9]. Although the majority of studies have used [C11]PIB [11] for this purpose, recent research has shown that [F18] labeled compounds, such as [F18]-flutemetamol, can bind to the cerebral amyloid deposits with the same affinity as [C11]PIB [31]. Therefore, we believe that the results obtained in this study with [F18]-flutemetamol mirror those that would have been found with the traditional method and represent a valid assessment of the brain amyloid load. When considered individually, all three biomarkers distinguished between patients and controls with equal

validity. However, when the biomarkers were used in combination discrimination improved, especially when a composite score including the three biomarkers is used (sensitivity/specificity 72/84%). An important issue is the set-up of specific cut-offs in order to proceed to a binary (normal/impaired) classification, as it is required by both the NIA-AA criteria and by the clinical practice. In order to be able to compare the three biomarkers with one another the cut-offs chosen were set up at the 10th percentile of the same control group. The resulting cut-offs were very similar with those provided by the literature for the PET-amyloid (>1.5) [31] and PET-FDG (>1) [34]. To the best of our knowledge no specific cut-offs are available for the N_HVOL but in a recent study on the ADNI database [40] the control group had a mean(SD) for the mean (left & right) hippocampus of 3558(408) mm³ which is very similar to the raw values of the controls from this study: 3540(391) mm³ (see Supplementary Table 2A–C).

When the NIA-AA guidelines were applied discrimination improved because 85% of patients and 87% of controls could be classified. For a majority of patients (62%), the method based on biomarkers was confirmatory of the cognitive status assessed using the neuropsychological battery. Indeed, most SCI patients were biomarker negative stage 0 (71%) and most MCI patients were biomarker positive and “due to AD” (56%). Interestingly, however, an important minority showed a pattern of impairment difficult to categorize. For instance, the number of amyloid-negative but neurodegeneration-positive (PET-FDG, N_HVOL or both) cases was not negligible, although it was similar in the patient and the control groups (14% of SCI and 15% of MCI versus 16% of controls). In contrast to amyloid-positive cases, most were either hypometabolic (seven) or had hippocampal atrophy (five) but rarely both (only two, both in the SCI group). Although according to NIA-AA criteria such patients are classified as either MCI “core” (conflicting results) if they have cognitive impairment or SNAP if they do not, their underlying pathology is not clear and is subject to debate [41–43]. Arguably, use of measures reflecting the distribution of AD-type pathology in this study may have concealed indications of non-AD pathology with a different distribution in these patients. According to the Dynamic model [44], one should expect MCI patients due to AD to have both hypometabolic associative areas and hippocampal atrophy. However, this was only the case for 13 out of 22 MCI due to AD patients (59%). This finding deserves further scrutiny, because it is difficult to attribute it exclusively to the lack of sensitivity of

the methods used. A clue to understanding this result may come from the observation that among the nine remaining MCI due to AD patients, seven had hippocampal atrophy alone and only two were exclusively hypometabolic. We can speculate that in incipient AD, it is easier to show a reduction in N_HVOL, especially in cases presenting as aMCI, whereas the involvement of association areas is patchier or unilateral and thus more difficult to determine with the PET-FDG measure used. Alternatively, it has also been suggested that subtypes of AD exist with predominant involvement of the mediotemporal lobe or of lateral association areas [45, 46]. Further work is needed to disentangle this issue. In the meantime, the corollary of this situation is a higher sensitivity for the method that included the two neurodegeneration biomarkers in combination (hippocampal atrophy OR hypometabolism), which is in agreement with the findings from ADNI studies [41].

Although a majority of patients had their clinical diagnosis confirmed by the biomarker assessment, the neuropsychological classification produced false negative (SCI patients with positive biomarkers) or false positive cases (MCI patients with negative biomarkers) in an important minority of patients (14 patients = 23%). In the first situation (six false negatives – 10%), the biomarker assessment provided useful information, because the SCI patients with positive biomarkers were far more often multimarker positive than the corresponding subjects from the control group, which emphasizes their higher risk of having a neurodegenerative disorder. One possible reason why these patients were missed by the neuropsychological examination is that five of the six patients had university level educational status and thus may have had a higher brain reserve with greater compensation capacities [47]. The second situation (8 false positives – 13%) occurred slightly more often than false negatives. False positive patients represented a quite similar percentage to the one of controls with abnormal cognitive function according to the method used (16%). They also had a pattern of cognitive deficits closer to that observed in controls in that they were very likely to be impaired for a single cognitive domain. It is, therefore, tempting to assume that these are indeed false positive cases and to take into account the possibility that impairment of a single domain (especially if other than memory) on the neuropsychological classification tends to produce too many false positive cases. Nevertheless, before making any conclusions, a more in-depth analysis of these cases is needed, either by considering their evolution or by expanding the biomarker analysis

to regions other than those typically involved in AD. Finally, the remaining three amyloid-positive but neurodegeneration-negative MCI patients may have AD-type pathology but were missed by the method we used to assess brain metabolism and hippocampal atrophy. Indeed, two of these patients had an AD score on PET-FDG close to the cut-off (0.95 and 0.85 for a cut-off of 0.99 and a control median at 0.45); the other one had a N_HVOL in the lower limit (between 10th and 15th percentiles).

In addition to determining whether a patient is cognitively impaired or not, the clinical classification also provides information about the pattern of impairment and, therefore, about the underlying neuropathology. For example, aMCI patients are more prone to have AD-type pathology and a multiple domain deficit should correspond to a higher degree of impairment with, arguably, a higher probability of finding positive biomarkers. The MCI multiple domain patients did indeed have more hippocampal atrophy than the MCI single domain patients, suggesting that their disease was more advanced, but no difference was observed for the remaining two biomarkers. This is not a fool-proof argument in favor of the hypothesis stated beforehand, because one should expect a more hypometabolic status from more extensive and presumably neocortical lesions in multiple domain MCI patients. These results, therefore, do not support a good capacity of the clinical diagnosis to assume extension of the underlying pathology. An important question for the clinician concerns the number of cases that can be reclassified when a second diagnostic method is applied on top of the first. We found that use of the biomarker method after clinical neuropsychological assessment led to a reclassification of 42% of patients, which represents a significant proportion for clinical practice because it represents slightly more than one non-demented patient in three.

Although the use of biomarkers provided a better classification than clinical assessment alone in this study, we should also highlight several limitations. The most important is the lack of confirmation of the diagnosis by anatomopathology or by follow-up. The cross-sectional character of the study did not allow the final diagnosis to be proven; hence, a longitudinal study is ongoing to disclose the final fate of these patients. The second limitation applies to many studies in this area including supposedly healthy controls. Although we made sure that control subjects were not cognitive complainers or suffered from conditions that might impair cognition we did not required from them a detailed account on the possible cases of dementia

in their families and this might have produced a selection bias. Another limitation concerns the method used and particularly the PET and MRI measures chosen. Although these markers are typically used in studies targeting incipient AD, they may not have revealed atypical localization of lesions and may have missed some non-AD dementias. Finally, a larger number of patients would have given more strength to the analysis, particularly for the less frequent situations, such as naMCI.

ACKNOWLEDGMENTS

We are grateful to the firm GE Healthcare Ltd. for having supplied with [18F]flutemetamol for the PET scan imaging according to a convention with our Clinic. We would also like to thank the anonymous reviewers who made very constructive suggestions that helped to significantly improve our manuscript.

Authors' disclosures available online (<http://www.j-alz.com/disclosures/view.php?id=2426>).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: <http://dx.doi.org/10.3233/JAD-140651>.

REFERENCES

- [1] Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E (1999) Mild cognitive impairment: Clinical characterization and outcome. *Arch Neurol* **56**, 303-308.
- [2] Reisberg B, Prichep L, Mosconi L, John ER, Glodzik-Sobanska L, Boksay I, Monteiro I, Torossian C, Vedvyas A, Ashraf N, Jamil IA, de Leon MJ (2008) The pre-mild cognitive impairment, subjective cognitive impairment stage of Alzheimer's disease. *Alzheimers Dement* **4**, S98-S108.
- [3] Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, Belleville S, Brodaty H, Bennett D, Chertkow H, Cummings JL, de Leon M, Feldman H, Ganguli M, Hampel H, Scheltens P, Tierney MC, Whitehouse P, Winblad B, International Psychogeriatric Association Expert Conference on mild cognitive impairment (2006) Mild cognitive impairment. *Lancet* **367**, 1262-1270.
- [4] Petersen RC, Negash S (2008) Mild cognitive impairment: An overview. *CNS Spectr* **13**, 45-53.
- [5] Ritchie K, Artero S, Touchon J (2001) Classification criteria for mild cognitive impairment: A population-based validation study. *Neurology* **56**, 37-42.
- [6] Fisk JD, Rockwood K (2005) Outcomes of incident mild cognitive impairment in relation to case definition. *J Neurol Neurosurg Psychiatry* **76**, 1175-1177.
- [7] Maioli F, Coveri M, Pagni P, Chiandetti C, Marchetti C, Ciarrocchi R, Ruggero C, Nativio V, Onesti A, D'Anastasio C, Pedone V (2007) Conversion of mild cognitive impairment to dementia in elderly subjects: A preliminary study in a memory

- and cognitive disorder unit. *Arch Gerontol Geriatr* **44**(Suppl 1), 233-241.
- [8] Stewart R (2012) Subjective cognitive impairment. *Curr Opin Psychiatry* **25**, 445-450.
 - [9] Jack CR Jr, Albert MS, Knopman DS, McKhann GM, Sperling RA, Carrillo MC, Thies B, Phelps CH (2011) Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 257-262.
 - [10] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 270-279.
 - [11] Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergstrom M, Savitcheva I, Huang GF, Estrada S, Ausen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Langstrom B (2004) Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol* **55**, 306-319.
 - [12] Trojanowski JQ, Vandevertichele H, Korecka M, Clark CM, Aisen PS, Petersen RC, Blennow K, Soares H, Simon A, Lewczuk P, Dean R, Siemers E, Potter WZ, Weiner MW, Jack CR Jr, Jagust W, Toga AW, Lee VM, Shaw LM, Alzheimer's Disease Neuroimaging Initiative (2010) Update on the biomarker core of the Alzheimer's Disease Neuroimaging Initiative subjects. *Alzheimers Dement* **6**, 230-238.
 - [13] Atiya M, Hyman BT, Albert MS, Killiany R (2003) Structural magnetic resonance imaging in established and prodromal Alzheimer disease: A review. *Alzheimer Dis Assoc Disord* **17**, 177-195.
 - [14] Jack CR Jr, Lowe VJ, Senjem ML, Weigand SD, Kemp BJ, Shiung MM, Knopman DS, Boeve BF, Klunk WE, Mathis CA, Petersen RC (2008) 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnesic mild cognitive impairment. *Brain* **131**, 665-680.
 - [15] Karow DS, McEvoy LK, Fennema-Notestine C, Hagler DJ Jr, Jennings RG, Brewer JB, Hoh CK, Dale AM, Alzheimer's Disease Neuroimaging I (2010) Relative capability of MR imaging and FDG PET to depict changes associated with prodromal and early Alzheimer disease. *Radiology* **256**, 932-942.
 - [16] Landau SM, Harvey D, Madison CM, Koeppe RA, Reiman EM, Foster NL, Weiner MW, Jagust WJ, Alzheimer's Disease Neuroimaging I (2011) Associations between cognitive, functional, and FDG-PET measures of decline in AD and MCI. *Neurobiol Aging* **32**, 1207-1218.
 - [17] Visser PJ, Verhey F, Knol DL, Scheltens P, Wahlund LO, Freund-Levi Y, Tsolaki M, Minthon L, Wallin AK, Hampel H, Burger K, Pirttila T, Soininen H, Rikkert MO, Verbeek MM, Spira L, Blennow K (2009) Prevalence and prognostic value of CSF markers of Alzheimer's disease pathology in patients with subjective cognitive impairment or mild cognitive impairment in the DESCRIPA study: A prospective cohort study. *Lancet Neurol* **8**, 619-627.
 - [18] Pike KE, Savage G, Villemagne VL, Ng S, Moss SA, Maruff P, Mathis CA, Klunk WE, Masters CL, Rowe CC (2007) Beta-amyloid imaging and memory in non-demented individuals: Evidence for preclinical Alzheimer's disease. *Brain* **130**, 2837-2844.
 - [19] Carrillo MC, Dean RA, Nicolas F, Miller DS, Berman R, Khachaturian Z, Bain LJ, Schindler R, Knopman D (2013) Revisiting the framework of the National Institute on Aging-Alzheimer's Association diagnostic criteria. *Alzheimers Dement* **9**, 594-601.
 - [20] American Psychiatric Association (2000) *Diagnostic and Statistical Manual of Mental Disorders, Text Revision (DSM-IV-TR®)*, American Psychiatric Association, Washington, DC.
 - [21] Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189-198.
 - [22] Manolio TA, Kronmal RA, Burke GL, Poirier V, O'Leary DH, Gardin JM, Fried LP, Steinberg EP, Bryan RN (1994) Magnetic resonance abnormalities and cardiovascular disease in older adults. The Cardiovascular Health Study. *Stroke* **25**, 318-327.
 - [23] Van der Linden M, Coyette F, Poitrenaud J, Kalafat M, Calicis F, Wyns C, Adam S, et les membres du GREMEM (2004) *L'épreuve de rappel libre/rappel indicé à 16 items (RL/RI-16) In L'évaluation des troubles de la mémoire: Présentation de quatre tests de mémoire épisodique (avec leur étalonnage)*, Van der Linden Mec, ed, Solal, Marseille.
 - [24] de Partz MP, Bilocq V, De Wilde V, Seron X, Pillon A (2001) *LEXIS: Tests pour l'évaluation des troubles lexicaux chez la personne aphasique*, Solal Editeurs, Marseille.
 - [25] Rouleau I, Salmon DP, Butters N, Kennedy C, McGuire K (1992) Quantitative and qualitative analyses of clock drawings in Alzheimer's and Huntington's disease. *Brain Cogn* **18**, 70-87.
 - [26] Morris JC, Mohs RC, Rogers H, Fillenbaum G, Heyman A (1988) Consortium to establish a registry for Alzheimer's disease (CERAD) clinical and neuropsychological assessment of Alzheimer's disease. *Psychopharmacol Bull* **24**, 641-652.
 - [27] FreeSurfer, Martinos Center for Biomedical Imaging, Boston, MA, USA, <http://surfer.nmr.mgh.harvard.edu/>
 - [28] Dale AM, Fischl B, Sereno MI (1999) Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* **9**, 179-194.
 - [29] Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM (2002) Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron* **33**, 341-355.
 - [30] Fischl B, van der Kouwe A, Destrieux C, Halgren E, Segonne F, Salat DH, Busa E, Seidman LJ, Goldstein J, Kennedy D, Caviness V, Makris N, Rosen B, Dale AM (2004) Automatically parcellating the human cerebral cortex. *Cereb Cortex* **14**, 11-22.
 - [31] Vandenberghe R, Van Laere K, Ivanoiu A, Salmon E, Bastin C, Triau E, Hasselbalch S, Law I, Andersen A, Korner A, Minthon L, Garraux G, Nelissen N, Bormans G, Buckley C, Owenius R, Thurfjell L, Farrar G, Brooks DJ (2010) 18F-flutemetamol amyloid imaging in Alzheimer disease and mild cognitive impairment: A phase 2 trial. *Ann Neurol* **68**, 319-329.
 - [32] Pmod Technologies Ltd Z, Statistical Parametric Mapping, <http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>
 - [33] Varrone A, Asenbaum S, Vander Borgh T, Booi J, Nobili F, Nagren K, Darcourt J, Kapucu OL, Tatsch K, Bartenstein P, Van Laere K, European Association of Nuclear Medicine Neuroimaging C (2009) EANM procedure guidelines for PET brain imaging using [18F]FDG, version 2. *Eur J Nucl Med Mol Imaging* **36**, 2103-2110.

- [34] Herholz K, Westwood S, Haense C, Dunn G (2011) Evaluation of a calibrated (18)F-FDG PET score as a biomarker for progression in Alzheimer disease and mild cognitive impairment. *J Nucl Med* **52**, 1218-1226.
- [35] Haense C, Herholz K, Jagust WJ, Heiss WD (2009) Performance of FDG PET for detection of Alzheimer's disease in two independent multicentre samples (NEST-DD and ADNI). *Dement Geriatr Cogn Disord* **28**, 259-266.
- [36] Jack CR Jr, Knopman DS, Weigand SD, Wiste HJ, Vemuri P, Lowe V, Kantarci K, Gunter JL, Senjem ML, Ivnik RJ, Roberts RO, Rocca WA, Boeve BF, Petersen RC (2012) An operational approach to National Institute on Aging-Alzheimer's Association criteria for preclinical Alzheimer disease. *Ann Neurol* **71**, 765-775.
- [37] MedCalc, MedCalc Software, <http://www.medcalc.org/index.php>
- [38] Herholz K, Salmon E, Perani D, Baron JC, Holthoff V, Frollich L, Schonknecht P, Ito K, Mielke R, Kalbe E, Zundorf G, Delbeuck X, Pelati O, Anchisi D, Fazio F, Kerrouche N, Desgranges B, Eustache F, Beuthien-Baumann B, Menzel C, Schroder J, Kato T, Arahata Y, Henze M, Heiss WD (2002) Discrimination between Alzheimer dementia and controls by automated analysis of multicenter FDG PET. *Neuroimage* **17**, 302-316.
- [39] Frisoni GB, Fox NC, Jack CR Jr, Scheltens P, Thompson PM (2010) The clinical use of structural MRI in Alzheimer disease. *Nat Rev Neurol* **6**, 67-77.
- [40] Mulder ER, de Jong RA, Knol DL, van Schijndel RA, Cover KS, Visser PJ, Barkhof F, Vrenken H, Alzheimer's Disease Neuroimaging I (2014) Hippocampal volume change measurement: Quantitative assessment of the reproducibility of expert manual outlining and the automated methods FreeSurfer and FIRST. *Neuroimage* **92**, 169-181.
- [41] Weiner MW, Veitch DP, Aisen PS, Beckett LA, Cairns NJ, Green RC, Harvey D, Jack CR, Jagust W, Liu E, Morris JC, Petersen RC, Saykin AJ, Schmidt ME, Shaw L, Shen L, Siu-ciak JA, Soares H, Toga AW, Trojanowski JQ, Alzheimer's Disease Neuroimaging I (2013) The Alzheimer's Disease Neuroimaging Initiative: A review of papers published since its inception. *Alzheimers Dement* **9**, e111-e194.
- [42] Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, Szoek C, Macaulay SL, Martins R, Maruff P, Ames D, Rowe CC, Masters CL, Australian Imaging B, Lifestyle Research G (2013) Amyloid beta deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: A prospective cohort study. *Lancet Neurol* **12**, 357-367.
- [43] Villain N, Chetelat G, Grassiot B, Bourgeat P, Jones G, Ellis KA, Ames D, Martins RN, Eustache F, Salvado O, Masters CL, Rowe CC, Villemagne VL, Group AR (2012) Regional dynamics of amyloid-beta deposition in healthy elderly, mild cognitive impairment and Alzheimer's disease: A voxelwise PiB-PET longitudinal study. *Brain* **135**, 2126-2139.
- [44] Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* **9**, 119-128.
- [45] Mukherjee S, Trittschuh E, Gibbons LE, Mackin RS, Saykin A, Crane PK, Alzheimer's Disease Neuroimaging I (2012) Dysexecutive and amnesic AD subtypes defined by single indicator and modern psychometric approaches: Relationships with SNPs in ADNI. *Brain Imaging Behav* **6**, 649-660.
- [46] Dickerson BC, Wolk DA, Alzheimer's Disease Neuroimaging I (2011) Dysexecutive versus amnesic phenotypes of very mild Alzheimer's disease are associated with distinct clinical, genetic and cortical thinning characteristics. *J Neurol Neurosurg Psychiatry* **82**, 45-51.
- [47] Tucker AM, Stern Y (2011) Cognitive reserve in aging. *Curr Alzheimer Res* **8**, 354-360.