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Chapter 5

Biomarkers in future

5.1
New research criteria for the diagnosis of AD applied in a memory clinic population

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Submitted

Abstract

Background
In the newly proposed research criteria for Alzheimer's disease patients are defined as having memory dysfunction in addition to either hippocampal atrophy, or abnormal CSF profile. This study applies the criteria in a memory clinic population, using clinical criteria as referent criterion.

Material and Methods
138 AD patients, 145 non demented subjects, 78 patients with other dementias and 91 patients with mild cognitive impairment (MCI) were included. Dichotomized medial temporal lobe atrophy (MTA) score on MRI and dichotomized CSF profiles (based on beta-amyloid1-42, Tau and phosphorylated Tau at threonine 181 levels) were used in combination with an episodic memory test to assess sensitivity, specificity and likelihood ratios (LR) of the newly proposed criteria and their components separately.

Results
We found specificities of 95% and 49% for comparison with non demented subjects and other demented patients respectively, with a sensitivity of 86% for AD. Specificity was highest (100% and 77% respectively, LR+ =48) when both MTA score and CSF profile were abnormal in addition to the episodic memory test, at the cost of a low sensitivity (48%).

Conclusion
The newly proposed research criteria for AD yield a good specificity for comparison with non demented subjects. When the type of dementia is clinically doubted, however, at least two supportive features should be considered (i.e. abnormal MTA score and CSF profile) in addition to memory impairment as core diagnostic criterion.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia. The clinical criteria that are commonly used for the diagnosis of probable AD, were proposed more than 20 years ago and largely depend on exclusion of other dementias [1]. Even in patients who have been followed up clinically for several years at expert research centres, the diagnostic accuracy is relatively low, with specificity of around 70% and sensitivity of 80% [4]. A definite diagnosis of AD can only be made at autopsy. The major histopathological hallmarks of AD are senile plaques, containing beta-amyloid42 (Aβ42) and neurofibrillary tangles with microtubuli-associated Tau protein. These pathological changes start in the medial temporal lobe and are present before the onset of clinical dementia [207, 208]. Research in the past decade has focussed on identification of AD pathology, using biomarkers. On MRI, atrophy of the medial temporal lobe is a marker of AD [209]. In cerebrospinal fluid (CSF), lower Aβ42 levels and elevated (phosphorylated) Tau protein levels can differentiate patients with AD from control subjects or patients with other neurological conditions with relatively high accuracy [7]. In planning of trials with disease-modifying treatments, especially in the earliest stages of the disease, it is of the utmost importance to include only those patients who are most certain to actually have AD. This is not only essential to maximize the chance of successful treatment but also to limit the exposure of potentially toxic therapies to those with AD. More importantly, those who have non-AD dementia or no dementia at all should be reliably excluded. In a recently published position paper new research criteria for the diagnosis of AD were proposed that would allow diagnosis related to neuropathological changes in AD, enabling intervention at an early or preclinical stage [58].The authors suggest to make use of supportive features on MRI, CSF and FDG-PET, that identify the AD-associated structural and molecular changes in the brain and their biochemical footprints, in combination with the core diagnostic criterion of episodic memory impairment. In this study we applied the above mentioned new research criteria, using MRI and CSF in addition to memory function, in our memory clinic population. FDG-PET was not routinely available. The newly proposed criteria were compared with the presently widely used clinical criteria according to the NINCDS-ADRDA in AD patients versus non demented controls and versus other dementias.

Materials and Methods

Study population

452 patients were recruited at the Alzheimer Center of the VU Medical Center (VUMC) between October 2000 and April 2007. The group comprised 138 AD patients according to the NINCDS-ADRDA criteria (135 probable AD, 3 possible AD), 78 patients with other dementias (43 Frontotemporal lobar degeneration (FTLD) according to the criteria of Neary and Snowden for FTLD [93], 7 Vascular dementia (VaD) according to the NINDS-AIREN criteria for VaD [9], 15 dementia with Lewy bodies (DLB) according to the criteria of McKeith et al for DLB [210], 4 corticobasal degeneration and 3 progressive supranuclear palsy according to previously published criteria [211-213], 1 Huntington disease and 1 hereditary Creutzfeldt Jacob disease confirmed genetically and 4 with undetermined aetiology of dementia) and 145 subjects without dementia (90 patients with subjective complaints, 55 other neurological or psychiatric disorders) and 91 patients with mild cognitive impairment (MCI) according to

the criteria of Petersen et al. [5, 214]. Patients underwent a standardized clinical assessment, including medical history, physical and neurological examination, and psychometric evaluation, laboratory tests, lumbar puncture (LP), EEG and brain MRI. Diagnoses were made by consensus in a multidisciplinary team. Follow-up data (mean follow-up time of 1.7±1.0 years) were available for 70 MCI patients: 35 remained stable, 30 progressed to AD. Five patients who progressed to other dementias were excluded from analysis (1 FTLD, 1 VaD, 1 DLB, 1 Parkinson's disease dementia, 1 alcoholic with vascular damage and frontoparietal atrophy on MRI). Psychometric evaluation, MRI and LP were performed within one year of the baseline diagnosis. The study was approved by the ethical review board of the VUMC and all subjects gave written informed consent.

Neuropsychological assessment

The neuropsychological test battery was designed to screen the major cognitive functions and included the Mini-Mental State Examination (MMSE) and the Visual Association Test (VAT)[177, 215]. The MMSE was used to assess dementia severity. For assessment of episodic memory the VAT was used. Patients are shown cue cards with an object and association cards with the previously seen object plus an interacting object and are asked to name each object. Subsequently, the cue cards are shown and patients are asked to recall the now missing interacting objects (score 0-12). The memory scores were dichotomized, with a score of 11 or 12 considered normal, and a score below 11 considered abnormal. Other tests used to diagnose MCI comprised the forward condition of the Digit Span to assess attention and the backward condition to assess working memory. In both cases an extended version with three trials per sequence length was used. The Trail Making Test was used in which part A provides a measure of mental speed and part B was used to evaluate executive functioning. In a test of category fluency patients had to produce as many animal names as possible in 60 seconds, assessing executive and language function.

CSF analysis

CSF was obtained by LP between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 12mL polypropylene tubes. Within two hours, CSF samples were centrifuged at 2100*g* for 10 minutes at 4˚C. A small amount of CSF was used for routine analysis, including total cells (leucocytes and erythrocytes), total protein and glucose. CSF was aliquoted in polypropylene tubes of 0.5 or 1ml, and stored at -80˚C until further analysis. CSF Aβ42, Tau and Tau phosphorylated at threonine-181 (P-Tau) were measured by commercially available sandwich ELISAs (Innotest® beta-amyloid1-42, Innotest® hTau-Ag and Innotest® Phosphotau $_{(181P)}$, Innogenetics, Ghent, Belgium). The inter-assay coefficient of variation for Aβ42 was 14%, 12% for Tau and 9% for P-Tau. The optimal cut off values for CSF Aβ42, Tau and P-Tau levels were set at data obtained in earlier studies, in which we applied a sensitivity of ≥ 85% for each individual biomarker in accordance with the Ronald and Nancy Reagan Consensus report after drawing Receiver Operating Characteristics curves. [57] The following cut off values were used: CSF Aβ42<495pg/mL, CSF Tau>356pg/mL and P-Tau>54pg/mL [64, 216]. A CSF profile combining Aβ42, Tau and P-Tau was constructed, which was abnormal when at least two of the three biomarkers were abnormal. The team involved in the diagnostic work-up was not aware of the results of the CSF analyses.

MRI analysis

MRI scans were made on a 1.0 Tesla scanner (Siemens Magnetom Impact Expert, Erlangen, Germany) and included a coronal T1-weighted 3D inversion-prepared gradient echo-sequence (168 slices, FOV 250mm, matrix 256x256; slice thickness 1.5mm, TE: 7ms, TR: 15 ms, TI 300 ms, flip angle 15 degrees). MTA was rated visually using a scale ranging from 0 (no atrophy) to 4 (severe atrophy) [209]. MTA scores of the left and right hippocampi were averaged [208]. In one patient only the left MTA score was available because right inferior temporal lobe was compressed by an epidermoidal cyst. Although brain MRI contributed to the diagnostic process by excluding other neurological diseases (e.g. brain tumour), MTA scores were not used in the diagnostic process. Two trained raters, who were blinded to clinical information, performed the MTA rating for this study. To determine reliability, 20 scans were scored twice (weighted Cohen's kappa for intrarater reliability>0.85 and interrater variability > 0.80). MTA scores were dichotomized according to age. Subjects<65 had an abnormal MTA score≥1, subjects≥65 and <75 had an abnormal MTA score≥1.5, and subjects≥75 had an abnormal MTA score≥2.

Operationalization of the new research criteria

The new research criteria for AD [58]were applied as follows. Patients were defined as having AD according to the new research criteria when there was evidence of episodic memory impairment based on an abnormal VAT-score and additionally positive evidence for the presence of Alzheimer pathology, as evident from abnormal MTA-score AND/OR abnormal CSF profile. To assess the components of the new research criteria separately, sensitivity, specificity and likelihood ratios (LR's) of VAT alone, VAT combined with MTA, VAT combined with CSF and abnormal VAT with abnormal MTA AND CSF profile were additionally determined.

Statistical analysis

For statistical analysis, SPSS version 14.0 (Chicago IL) was used. One way Analysis of Variance (ANOVA) with post hoc Bonferroni tests was used to compare continuous variables between diagnostic groups. Frequency distributions for sex were compared with chi-squared tests. Sensitivities (= AD patients with abnormal test scores / total AD patients), specificities (= other or non demented subjects with normal test scores / total of other or non demented subjects) and likelihood ratios (LR+ =sensitivity/1-specificity, LR- =1-sensitivity/specificity) were calculated of the new criteria, VAT alone, VAT combined with MRI, VAT combined with CSF and VAT with both MRI and CSF abnormal. Sensitivities, specificities and LR's were calculated for AD versus other dementias, AD versus non-demented controls and AD versus other or no dementia (previous groups combined) using the diagnosis made in multidisciplinary setting according to clinical criteria as referent criterion (i.e. gold standard). 95% confidence intervals of sensitivities and specificities were calculated with the formula $\pm 1.96\sqrt{((s \times (1-s))/n)}$, in which s represents sensitivity or specificity and n the number of patients. In an additional analysis, sensitivity, specificity and likelihood ratios of the new criteria among MCI patients were investigated. Follow-up diagnoses were used as referent criterion.

Results

The demographic characteristics of the diagnostic groups are presented in table 1. No differences were found for sex. The no dementia group was younger than the AD, other dementias and MCI groups. The other dementias group was younger than the MCI group. According to the baseline MMSE AD and other demented patients were moderately demented. MCI patients scored in between demented and non-demented subjects. There were group differences for memory score, MTA-score and CSF profile, AD patients having most abnormal scores, the no dementia group having normal scores and the other dementia and MCI groups scoring mostly intermediate (see table 1 for details). The specificity, sensitivity and likelihood ratios of the new research criteria are presented in table 2 and 3. The new criteria had good sensitivity (86%), and almost perfect specificity for AD compared to no dementia (95%), which is reflected by the high positive and low negative likelihood ratios. When compared with patients with other dementias, specificity was modest (49%). Subsequently sensitivities and specificities were specified for components of the new criteria. There was a good specificity (86%) and sensitivity (93%) of the memory score alone compared with controls, but as memory impairment is a characteristic of most types of dementia, specificity (33%) was poor in comparison with other demented patients. The specificity for both comparisons increased when either MTA score (98%) or CSF profile (97%) were added to the memory score. This comes at the expense of lower sensitivity, however, which decreased most for memory score combined with MTA score (54%) and less for memory score combined with CSF profile (80%). Finally, when memory score, MTA score and CSF profile were all abnormal specificity for both comparisons (i.e. versus other and non demented patients) was best (100% and 77%) with a positive likelihood ratio of 48, while the sensitivity for AD remained modest (48%). MCI patients were analysed separately according to the diagnosis at follow-up. Sensitivity, specificity and likelihood ratios are presented in table 4. The specificity of the revised research criteria is lower for MCI AD than for AD (69% versus 97%), but increases considerably when both MTA score and CSF profile are added to 97%.

Discussion

This study compared the newly proposed research criteria for AD with clinical diagnoses in a memory clinic population including MCI patients. We found an almost perfect specificity of 95% for comparison with non demented subjects and a modest specificity of 49% compared with other demented patients, while the specificity for stable MCI patients was 69%. There was a good sensitivity of 86% for AD patients and the sensitivity for MCI patients who progressed to AD was 77%.The specificity represents the percentage of patients without AD and normal test scores, correctly labelled as non AD (specificity = other or non demented subjects with normal test scores / total of other or non demented subjects). The newly proposed research criteria for AD stress the importance of a good specificity, in order to exclude patients with non AD dementia and limit the exposure of possibly toxic therapies to those who have pure AD. Sensitivity represents the percentage of AD patients who have abnormal test scores, justly labelled as AD (sensitivity =AD patients with abnormal test scores / total AD patients). Pure AD patients are most likely to benefit from medication,

directed against AD neuropathology. The specificity in comparison with non-demented subjects is almost perfect, which supports use of these criteria for selection of therapeutic trials in AD. The specificity for other dementias, however, is modest. This is in agreement with previous studies, showing overlap of memory dysfunction, abnormal MTA-score and CSF profile between patients with AD and patients with other dementias [7, 217, 218]. This overlap could partly be explained by the fact that most demented patients pathologically have mixed disease. Thus, although the diagnoses made during life fulfil established clinical criteria, post mortem examination often shows more pathologies than one. This mixed pathology may be better picked up by biomarkers like CSF and MRI. When in addition to memory function either MTA score or CSF profile are abnormal specificity increases (from 33% to 58% and 67%), but when both MTA score and CSF profile are abnormal in addition to memory function, specificity in comparison with other dementias reaches 77%. Thus, in case of clinical doubt about the type of dementia, a combination of memory impairment as core diagnostic criterion and at least two supportive features should be considered, instead of one supportive feature that is presently proposed in the new criteria. The specificity for AD in MCI patients is lower than we expected. This may be explained by the limited follow-up time of 1.7 years, which may not be long enough to detect all incipient AD cases in our MCI group. The lower specificity may mean that some patients had AD according to the new research criteria, when in fact they did not convert to AD within the study period. It is possible that the new criteria already recognized AD, when clinical follow-up was not yet long enough to diagnose AD according to the clinical criteria. Thus a longer follow up time may increase the specificity. The lower sensitivity for AD in MCI patients especially for the combination of memory and MTA score may be explained by the relatively young patient population, as young patients are known to have less prominent MTA. [219] The fact that young AD patients have less prominent MTA may also explain why the sensitivity of memory score & MTA is lower than the sensitivity of memory score & CSF. One might argue, that patients with other dementias (e.g. FTLD, VaD and DLB) clinically, do not have AD and that it seems artificial to subject them to criteria for AD. These patients fulfil previously published clinical criteria that could identify these patients in the first place and subsequently exclude them from this study. On the other hand, in clinical practice, the difference between patients with AD and FTLD, VaD or DLB is not always obvious. We feel that the application of the newly proposed criteria to various other dementias gives a good insight in the usability of these criteria in a memory clinic population. A possible limitation of our study is our measure of memory impairment. We used the visual association test (VAT), which provides a measure of immediate memory. According to the new research criteria, a delayed recall test should be used to assess early episodic memory impairment related to AD. Unfortunately we do not have sufficient delayed recall data for our memory clinic population. This may have influenced the results, especially in early AD, when memory impairment is in the foreground. The new research criteria also mention, however, that (early) AD patients show reduced benefit from cueing at recall, which should be used for measuring memory impairment. VAT is a specifically cued task. It has been demonstrated that the VAT with testing associative learning, is highly specific and sensitive for detecting (early) AD and excluding non-AD subjects. Therefore, we feel that the VAT is a strong alternative for measuring memory impairment.

Table 1

	AD	Other dementias	No dementia	MCI	p-value
Number	138	78	145	65	
Sex F/M	70/68	27/51	67/78	30/35	0.15
Age (years)	68 (8)	66 (9)	60 (10)	70 (8)	<0.01 [a]
MMSE#	21.5 (4.6)	22.7 (5.7)	28.3 (1.8)	26.3 (2.7)	<0.01 [b]
Memory-score##	4.7 (3.5)	7.5 (4.0)	11.4 (1.3)	8.5 (3.3)	<0.01 [c]
MTA-score	1.4 (0.9)	1.4 (1.1)	0.3 (0.5)	0.9 (0.9)	<0.01 [d]
Aβ42 (pg/ml)	443 (171)	706 (294)	817 (223)	551 (222)	<0.01 [e]
Tau (pg/ml)	768 (466)	458 (359)	300 (206)	604 (466)	<0.01 [f]
P-Tau (pg/ml)	89 (39)	57 (28)	48 (22)	78 (41)	<0.01 [g]

Demographics of memory clinic population. Data are presented as mean (SD). Aβ42=beta-amyloid1-42, P-Tau= phosphorylated Tau at threonine 181, MTA=mean medial temporal lobe atrophy score. ## Memory score based on visual association test. # Baseline MMSE was missing for 1 non-demented subject, 2 AD patients and 5 other demented patients. [a] no dementia<AD=other dementias=MCI; [b] no dementia>MCI>AD=other dementias; [c] no dementia >MCI=other dementias>AD; [d] no dementia<MCI<other dementias=AD; [e] no dementia>other dementias>MCI>AD; [f] no dementia<other dementias=MCI<AD; [g] no dementia=other dementias<MCI=AD.

Another possible limitation is the fact that MRI was part of the diagnostic work up of our memory clinic population, e.g. to exclude other neurological diseases like brain tumour [4]. Using MRI during the diagnostic process may have slightly influenced our results, as the presence of MTA may have influenced the physicians to a diagnosis of AD. We therefore cannot entirely exclude the possibility of circular reasoning. In a similar way, the clinical diagnosis made in multidisciplinary team makes use of memory scores.

Table 3

	AD versus no dementia (n=145)		AD versus other dementia (n=78)		AD versus other or no dementia (n=223)	
	LR+	LR-	LR+	LR-	LR+	LR-
New criteria (memory + MTA OR CSF)	17.2	0.15	1.7	0.29	4.10	0.18
Memory	6.6	0.1	1.4	0.2	2.3	0.1
Memory + MTA	27	0.5	1.3	0.8	3.4	0.6
Memory + CSF	27	0.2	2.5	0.3	6.2	0.2
Memory + MTA AND CSF	48	0.5	2.1	0.7	6	0.6

Likelihood ratios of revised AD criteria in a memory clinic population. Memory=visual association test score abnormal<10, MTA-score abnormal ≥1for subjects<65, ≥1.5 for subjects ≥ 65 and <75, and ≥2 for subjects ≥75. CSF abnormal when two of the three biomarkers abnormal (cut off values: CSF Aβ1-42<495, CSF Tau>356, P-Tau>54 pg/mL).

This tool is also part of the new AD criteria. Thus, using this clinical diagnosis as referent criterion may have led to circularity of the validation strategy. This is a largely descriptive study, describing how many patients attending a memory clinic meet both sets of criteria and how the criteria overlap.

Table 4

	MCI-AD (n=30) versus MCI-stable (n=35)			
	Specificity	**Sensitivity**	**LR+**	**LR-**
New criteria (memory + MTA OR CSF)	24/35= 69% (54-84)	23/30= 77% (62-92)	2.44	0.33
Memory	19/35= 54% (37-71)	24/30= 80% (66-94)	1.75	0.37
Memory + MTA	32/35= 91% (82-100)	8/30= 27% (11-43)	3.1	0.80
Memory + CSF	26/35= 74% (59-89)	22/30= 73% (57-89)	2.9	0.36
Memory + MTA and CSF	34/35= 97% (91-103)	7/30= 23% (8-38)	8.03	0.79

Specificity, sensitivity and LR's of revised AD criteria in MCI patients.

In a first effort to demonstrate the possible use of the new research criteria in a clinical sample. Future studies may be devised to fine-tune the research criteria by assessing the value of combined measures in ROC analyses. A reduction of glucose metabolism as seen on PET in bilateral temporal parietal regions and in the posterior cingulate is a commonly described diagnostic criterion for AD [220]. Unfortunately, we were not able to include FDG-PET scan data in our study, because of the low number of available scans. Both MTA on MRI and CSF biomarkers have been shown to discriminate AD from controls and other dementias with comparable accuracy as FDG-PET [220]. Moreover, in general they are more easily available and less time consuming in clinical practice than PET scans. Alas, the lack of neuropathological confirmation of our patient population limits the ultimate interpretation towards the final diagnosis. Despite the lack of FDG-PET scan and neuropathological data, however, this study gives a good insight into the categorization of a memory clinic population after applying the newly proposed criteria. The ultimate goal of the new research criteria is to be able to apply specific anti AD therapy to AD patients as early in the disease process as possible. Therefore, we feel that a high specificity is more important than a high sensitivity. The newly proposed AD criteria yield a high specificity in subjects with no dementia. When there is clinical doubt about the type of dementia, two (instead of the presently proposed one) supportive features should be abnormal in addition to the core criterion of memory impairment to maintain specificity towards other dementias.

Table 2

	AD versus no dementia (n=145)	AD versus other dementia (n=78)	AD versus other or no dementia (n=223)	Sensitivity (n=138 AD patients)
New criteria (memory + MTA OR CSF)	138/145= **95%** (91-99)	38/78= **49%** (38-60)	176/223= **79%** (74-84)	119/138= **86%** (80-92)
Memory	125/145= **86%** (80-92)	26/78= **33%** (23-43)	151/223= **68%** (62-74)	128/138= **93%** (89-97)
Memory + MTA	142/145= **98%** (96-100)	45/78= **58%** (46-70)	187/223= **84%** (79-89)	75/138= **54%** (46-62)
Memory + CSF	141/145= **97%** (94-100)	53/78= **68%** (58-78)	194/223= **87%** (83-91)	110/138= **80%** (73-87)
Memory + MTA AND CSF	145/145=**100%** (-)	60/78= **77%** (68-86)	205/223= **92%** (88-96)	66/138= **48%** (40-56)

Specificity of revised AD criteria in a memory clinic population. AD=Alzheimer's disease. MTA=medial temporal lobe atrophy. Memory = visual association test score abnormal<10, MTA-score abnormal≥1 for subjects<65, ≥1.5 for subjects≥65 and <75, and ≥2 for subjects ≥75. CSF abnormal when two of the three biomarkers abnormal (cut off values: CSF Aβ42<495, CSF Tau>356, P-Tau-181>54 pg/mL). 95% confidence intervals are shown in between brackets. Note that specificity=(other or non demented subjects with normal test scores) / (total of other or non demented subjects) and sensitivity=(AD patients with abnormal test scores) / (total AD patients). Thus another control group (i.e. no or other dementia) does not change sensitivity.