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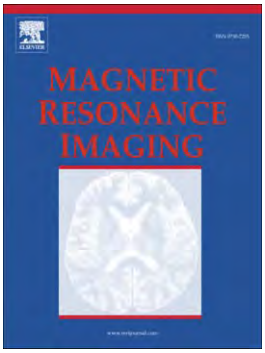

Magnetic resonance spectroscopy and brain volumetry in mild cognitive impairment. A prospective study

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Magnetic resonance spectroscopy and brain volumetry in Mild Cognitive Impairment. A prospective study.

ABSTRACT

OBJECTIVE: To assess the accuracy of magnetic resonance spectroscopy (1H-MRS) and brain volumetry in mild cognitive impairment (MCI) to predict conversion to probable Alzheimer's disease (AD).

METHODS: Forty-eight patients fulfilling the criteria of amnestic MCI who underwent a conventional magnetic resonance imaging (MRI) followed by MRS, and T1-3D on 1.5 Tesla MR unit. At baseline the patients underwent neuropsychological examination. 1H-MRS of the brain was carried out by exploring the left medial occipital lobe and ventral posterior cingulated cortex (vPCC) using the LCModel software. A high resolution T1-3D sequence was acquired to carry out the volumetric measurement. A cortical and subcortical parcellation strategy was used to obtain the volumes of each area within the brain. The patients were followed up to detect conversion to probable AD.

RESULTS: After a 3-year follow-up, 15 (31.2%) patients converted to AD. The myo-inositol in the occipital cortex and glutamate+glutamine (Glx) in the posterior cingulate cortex predicted conversion to probable AD at 46.1% sensitivity and 90.6% specificity. The positive predictive value was 66.7%, and the negative predictive value was 80.6%, with an overall cross-validated classification accuracy of 77.8%. The volume of the third ventricle, the total white matter and entorhinal cortex predict conversion to probable AD at 46.7% sensitivity and 90.9% specificity. The positive predictive value was 70%, and the negative predictive value was 78.9%, with an overall cross-validated classification accuracy of 77.1%. Combining volumetric measures in addition to

the MRS measures the prediction to probable AD has a 38.5% sensitivity and 87.5% specificity, with a positive predictive value of 55.6%, a negative predictive value of 77.8% and an overall accuracy of 73.3%.

CONCLUSION: Either MRS or brain volumetric measures are markers separately of cognitive decline and may serve as a noninvasive tool to monitor cognitive changes and progression to dementia in patients with amnestic MCI, but the results do not support the routine use in the clinical settings.

Key words: Mild cognitive impairment; Alzheimer's disease; Magnetic resonance spectroscopy; Brain volumetry

INTRODUCTION

The amnestic subtype of MCI (Mild cognitive impairment) is usually caused by a degenerative etiology, and most frequently represents a prodromal stage of AD (Alzheimer's disease). Form an epidemiologic perspective, the rates of progression to AD range from 6 to 10% per year but in the referral clinical setting the rates are higher (10-15% per year)[1] . The definitive diagnosis of AD can only be based on the combination of symptoms plus histopathology, with the presence of large number of neuritic plaques and neurofibrillary tangles in brain tissues[2]. The search for noninvasive diagnostic methods has been of considerable interest, because, apart from allowing detection of those who develop AD, they will serve to monitor changes and assess the effects of drugs in AD [3].

Atrophy of the hippocampus, and entorhinal cortex as well, are frequent prodromal signs of AD in subjects with MCI[4-8] . Whole brain, cortical, and ventricular volumes have also shown to be associated with progression to AD, although the volume measurements from MR images have relatively low accuracy in the prediction of AD[9-12] .

Proton magnetic resonance spectroscopy (1H-MRS) is one of the techniques used to assess potential disruptions of neuronal integrity and associated neurochemical dysregulations. MRS provides additional biochemical information, which can be useful to determine the clinical stratification for a specific patient. Myo-Inositol (mI) is considered a glial marker, and as a possible degradation product of myelin and osmolyte or regulator of cell volume. Choline (Cho) is a constituent of phospholipids metabolism of cell membranes and reflects cell proliferation. Creatine (Cr) plays an important role in brain energy system (ATP), a marker of brain metabolism. The N-acetylaspartate (NAA) is a marker of neuronal and axonal density and mitochondrial functioning. Glutamate (Glu) is the primary excitatory neurotransmitter in the brain. Studies have shown that the ratio of N-acetylaspartate/creatine can predict with high accuracy conversion from MCI to AD [13-15] . Also increased mI/Cr and decreased NAA/Cr ratios correlated

well with the severity of AD [16]. Hitherto studies suggest that MRS combined with measurements of brain volumes can provide neuroimaging markers of disease progression .

The purpose of this study is to explore whether the changes in brain volumes and metabolite levels are associated with cognitive decline and the development of dementia in amnestic-MCI patients over time. We hypothesize that MRS and brain volumetry can be useful predictors of cognitive deterioration in amnestic- MCI patients.

PATIENTS AND METHODS

The initial cohort was composed of 52 patients with amnestic MCI who were followed for a mean period of 3 years (standard deviation [SD]: 6.7 months). However, 4 subjects were discarded due to problems of movement or processing. Therefore, 48 patients (30 female and 18 male) with amnestic MCI were finally included in the study. All of them underwent a MRI brain volumetry assessment, MRS and clinical examination at baseline were included in a longitudinal study. Table 1 lists the main baseline demographic variables and the results of memory tests and scales. The mean age of the cohort was 74.4 years (SD: 6.9). The mean age was 73.7 (SD 7.1) years in women and 75.2 (SD 6.7) years in men. After a mean follow-up of 36 months, 15 (31.2%) patients out of 48 converted to probable AD according to the NINCDS-ADRDA group criteria, and none of them reverted to normality. There were 9 women and 6 male in the group of converters and 21 women and 12 male in the group of non-converters. Age did not differ significantly by gender (F=0.51, p=0.476), but converters were significantly older than non-converters (mean age: 78 (SD 5.5) years for converters vs 75 (SD 4.6) years for non-converters (F=8.34; P=0.006). No differences were seen in the male/female ratio proportion (Chi-Square, p=0.528), Sex has a significant effect on the volume of the white (F= 29.79; P= <0.001) and gray matter (F=28.14; P <0.001) (males have significant larger volumes in comparison with females). Age has a significant (negative) correlation with the total amount of gray matter. (r Pearson = -0.40, p = 0.005).

Conversion to dementia of probable Alzheimer type was based on the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) group criteria [17] . Informed consent was obtained from the participants before their inclusion in the study. The study protocol was approved by the ethical review board of the regional health authority.

Neuropsychological examination.

The patients include here were referred by general practitioners because of memory complaints which were corroborated by relatives or caregivers. The were first screened for memory impairment with the Memory Impairment Screen (MIS) [18], a simple delayed recall test. At baseline, the patients underwent the following neuropsychological examination: the Mini-Mental test (Spanish version with a maximum possible score of 35 points) [19], the Blessed Dementia Rating Scale, the clock drawing test, the Geriatric Depression Scale and the Rey Auditory Verbal Learning Test (RAVLT) delayed recall. The patients included in this study must score 5 or lower in the MIS, 0.5 in the CDR and a score in the Mini-Mental higher than 21 points. The cut-off points for the RAVLT 20 min delayed recall were as follows: ≤4 for patients aged up to 69 and ≤3 for patients aged 70 and older. Those with depressive symptoms (11 points or higher in the Geriatric Depression Scale) were re-evaluated after antidepressant treatment.

Magnetic Resonance Imaging.

MRI was performed on all patients. For the volumetric measurements, a three dimensional (3D) high-resolution whole-brain gradient-echo T1-weighted sequence was obtained on a 1.5 Tesla system (Signa HD, 12.x scanner; General Electric Medical Systems, Milwaukee, WI) using an 8-channel head coil. The acquisition protocol included the following parameters: 122 coronal slices, TR =

9.05 ms, TE = 1.72 ms, 1.5 mm slice thickness with no inter-slice gap, acquisition matrix = 256 x 256, flip angle = 20^{o} and voxel size = 0.86 x 0.86 x 1.5 mm. After acquisition, all images were reviewed by a radiologist and a computer engineer, who were blind to clinical subgroups, in order to ensure data quality. Additionally, a T1 axial, a T1 sagittal, a T2 and a fluid-attenuated inversion recovery sequences were also acquired with clinical purposes.

Magnetic Resonance Spectroscopy.

For the quantitative regional analysis, a sagittal and axial T1 and axial T2-weighted image was used to locate a voxel or volume of interest (2 x 2 x 2 cm). These areas of exploration were the left medial occipital lobe and posteriomedial parietal cortex. (Figs. 1A and 1b). 1H-MRS was performed using a short echo time of 35 milliseconds and a repetition time of 2000 milliseconds, and 128 accumulations were gathered using a single-voxel stimulated- echo acquisition-mode localization sequence with a spin-echo technique of selective excitation with and water suppression. The mode of spectral acquisition was probe-p (PRESS technique). The pure metabolite signal was spoiled, zero-filled, and Fourier transformed to produce a spectrum, scaled, drawn onto a 512-by-512 image, and stored as an image in the system database. Every spectrum was automatically fitted to four peaks corresponding to levels of N-acetyl-aspartate (NAA), 2.02 ppm; total creatine (Cr), 3.03 ppm; choline-containing compounds (Ch), 3.23 ppm; glutamate + glutamine (Glx), 2.1 to 2.55 ppm, and myo-Inositol (mI), 3.56 ppm. We also obtained the peak amplitude of the metabolites relative to Creatine. For this purpose we used the algorithms provided by the manufacturer with the following steps: 1. Setting a global frequency fit parameter. 2. Performing line width and line shape enhancement by appropriate apodization of the time domain signal. 3. Fourier transformation of the signal to the appropriate frequency resolution and number of points. 4. Calculation of a baseline correction from the frequency domain signal. 5. Curve fitting the desired regions of the frequency domain signal.

The postprocessing of data was done with the LCModel software, version 6.2-0 [20]. Concentration values were not corrected for contributions by cerebrospinal fluid or for small reductions in the numeric values because of residual T1 and T2 relaxation effects.

We also obtained the ratios of the metabolites relative to Cr. Spectra were rejected and repeated in the following cases: line width >10 Hz, line shape asymmetrical after eddy current correction and the presence of artifacts. Data fits with % SD >20 from the Cramér-Rao inequality were eliminated.

Both areas we examined showed excellent reproducibility in two previous studies of test-retest reliability carried out with the same clinical scanner in AD patients [21,22]. For the NAA/Cr ratios, the α (the interclass correlation coefficient) value was 0.93 and 0.95 in the posteromedial bilateral parietal lobe respectively, and 0.89 and 0.87 in the left medial occipital lobe.

Brain Volumetry.

The T1-3D images were used for the assessment of the brain volumes. After the qualitative quality control of the acquired images, they were blinded and sent to a workstation for analysis and post-processing. Before performing the volumetric measurements, all images were improved using a combination of two spatial filters. First, a non-local filter [23] was applied to minimize the random fluctuation of the MR signal due to the thermal noise. This is especially relevant when semiautomatic quantitative measurements are being considered. The resulting volumes showed a significant higher signal-to-noise ratio when compared with the original data. Then, images were also corrected for intensity heterogeneities which are usually present in MR images and depend on the sensitivity of the coils. A non-parametric method based on the minimization of an entropy-related function cost [24] was applied on each image to produce a non-biased corrected volume.

The Freesurfer software package (http://surfer.nmr.mgh.harvard.edu/) was used to perform the parcellation and the volumetric measurement of the

different brain areas. The method included several steps to extract white and gray matter surfaces and to compute the volume of cortical and subcortical areas segmented to allow labeling and identifying the regions. These areas were labelled defining a minimization cost function based on a combination of the probabilistic template location, image intensities and local relationships between cortical and subcortical regions. For the purposes of this study we included volumes from the following structures: total gray matter, total white matter, frontal lobe, temporal lobe, parietal lobe, occipital lobe, lateral ventricles, third ventricle, fourth ventricle, hippocampus, globus pallidus, amygdala, thalamus, caudate nucleus, putamen, and cerebellum. All these structures were independently analyzed bilaterally (right and left). The anatomic accuracy of the gray and white matter parcellation was checked qualitatively by a trained neuroradiologist and a computer engineer in order to detect faults in the segmentation process. Four subjects were excluded from the initial sample due to misregistration artifacts in the temporal lobe region only for volumetry purposes.

Clinical follow-up

The patients were followed up and re-evaluated every 6 months or earlier to determine whether they had developed probable AD-type dementia according to the NINCDS-ADRDA group criteria [17].

STATISTICS

All statistic tests were performed with the SPSS statistical package. Data derived from metabolite concentration and brain volumes were included in a statistical model alongside demographic and clinical variables. In order to minimize the risk of obtaining results related with macroscopic variability of the data (for example due to differences between males and females), all volumes were normalized by the total intracranial volume of each participant. This volume was computed as the sum of the gray matter, white matter and the cerebrospinal fluid. As age was statistically different between groups of

converters and non-converters (F=8.34; p=0.006), it was included as a covariate of interest in the model. Different tests were then performed to identify the automated measurements that best discriminated both groups of patients. For the ANOVA test, the assumptions of normality and homoscedasticity were tested. Homoscedasticity was tested by inspecting the Q-Q plots and by carrying out a Levene test. The Kolmogorov-Smirnov test was used to check the normality of the data. None of the considered variables in the study violated these assumptions.

In order to determine which variable (or combination of variables) was the best predictor of conversion to AD, we used a Fisher Linear Discriminant Analysis (LDA). Three different LDA classifiers were tested, including a classifier with only variables from spectroscopy, a classifier with volumetric variables and a classifier with both, spectroscopic and volumetric measurements. All Fisher LDA classifiers were constructed and evaluated using a cross-validation strategy.

RESULTS

The ANCOVA model that included the brain volumetric measures after normalizing the values by the total intracranial volume showed that the most significant variable was the volume of the third ventricle (F= 4.382, p= 0.042). With spectroscopy we found statistically significant variables for converters and non-converters: myo-inositol in occipital cortex (F= 6.332, p= 0.016); and the glutamate/creatine ratio (F=4.178, p=0.047) and glutamate + glutamine (Glx) in the ventral posterior cingulate cortex (F= 4.128, p= 0.049) (Table 2). Volume measurements are given in table 3.

After the individual evaluation of each variable using an ANCOVA approach, a LDA analysis was applied to determine which combination of variables is the best predictor for converting to AD. Considering the brain volumetry assessment, the following variables represented the greatest percentages of correct prediction: 3rd ventricle, entorhinal cortex and total white matter. The classifier constructed with these three variables predicted

conversion to probable AD at 46.67% sensitivity and 90.91% specificity, with a positive predictive value of 70.00% and a negative predictive value of 78.95%. The cross-validated accuracy of classification was 77.08%. myo-inositol in occipital cortex, glutamate/creatine ratio and glutamate + glutamine (Glx) in the posterior cingulate cortex predict conversion to probable AD at 46.15% sensitivity and 90.63% specificity, with a positive predictive value of 66.67% and a negative predictive value of 80.56%. The cross-validated accuracy of classification was 77.78%. Finally a unique LDA classifier including spectroscopic and volumetric variables (the same as in the previous single-modality LDA classifiers) was computed, achieving a prediction to probable AD at 38.46% sensitivity and 87.50% specificity, with a positive predictive value of 55.56%, a negative predictive value of 77.78% and an overall accuracy of 73.33%. The three proposed classifiers were also tested with the receiver operator characteristic (ROC) method, showing an area under the curve (AUC) of 0.78 for the volumetric classifier, of 0.81 for the spectroscopic classifier and of 0.78 for the combined classifier (Fig. 2).

DISCUSSION

In our study, we have found that baseline third ventricle volume, Myo-inositol in occipital cortex, the Glutamate/Creatine ratio, and Glutamate + Glutamine (Glx) levels in the posterior cingulate cortex in amnestic MCI predict with high risk of early conversion to AD in comparison with non-converters.

On the light of these results, it seems that MRS has more influence on the variable conversion to Alzheimer than volumetry (especially Myo-inositol in occipital cortex, the Glutamate/Creatine ratio (Glu/Cr), and Glutamate + Glutamine (Glx) levels in the posterior cingulate cortex). In the case of using a standardized total intracranial volume (TIV) and covariant age data, the only anatomical area showing a significant relationship with the variable conversion was the third ventricle volume.

With regard to MRS measurements, the myo-inositol in the occipital cortex + Glx in the posterior cingulate cortex, predicted with high specificity but

low sensitivity the conversion of MCI to probable Alzheimer's disease. It is generally observed that the negative predictive value is quite high but the positive predictive value is low.

Several studies have shown the usefulness of 1H MRS in the early detection of AD [13,15, 25-27]. 1H MRS has also detected biochemical abnormalities preceding brain atrophy and cognitive decline [28]. None of them included the levels of glutamate as predictor. In this study the accuracy of prediction of MRS was lower than in our two previous studies [13, 15] and comparable to volumetry, however the sample size is smaller and the follow-up period is shorter. The combination of MRS and volumetry was not superior to these techniques used individually.

Structural neuroimaging has also been validated as a tool to detect and monitor progression of AD. Tissue volumes in the central nervous system, and in particular changes in volume over time, are sensitive markers of disease progression. Manual segmentation to measure the hippocampal volume is recognized as the gold standard [29]. It has been found that atrophy of mesial temporal structures, such as the hippocampus and entorhinal cortex is predictive of progression to AD [30]. Using semi-automated segmentation threshold track, Jack et al. described a sensitivity of 82% and 80% specificity for discriminating AD patients in the control group in a study of 220 individuals [31]. Serial volumetric measurements in the prediction of AD and in evaluating the effectiveness of therapeutic regimens in high risk populations, particularly those with MCI, were also described. A study of 129 patients indicated that the hippocampal volume within 3 years follow-up revealed atrophy rates 10 times higher in patients with mild or moderate dementia compared with normal controls [4]. Another study found that rates of percentage reduction in brain volume, ventricles, hippocampus and temporal horn are more significant in patients with MCI who develop dementia compared to those who remained stable (32). Structural MRI alone has also proven insufficient to predict early AD and additional biomarkers are needed in combination to make reliable predictions in MCI (33). Myo-inositol reflects glial activation or

neuroinflammation associated with neuronal degeneration, and it is a sensitive marker reflecting pathological changes in MCI and AD [25, 34]. Kantarci et al [35] , studied subjects with microtubule-associated protein (MAPT) mutations. Their results indicated that the MI/Cr ratio, a possible index of glial activation, precedes the decrease of NAA/Cr, a neuronal integrity and hippocampal atrophy.

When analysing metabolite levels in the whole sample while controlling for age and gender, we observed that all metabolites were correlated with age. N-acetylaspartate, glutamate and glutamate+glutamine and their ratios to creatine showed a negative correlation (increase in age with a decrease in metabolite levels and vice versa), while the remaining metabolites, such as myo-inositol and choline, show a direct correlation. A decrease in glutamate and glutamate+glutamine over time, which is associated with a certain cognitive deterioration, was found in Alzheimer´s Disease [36] . Decreased levels of N-acetylaspartate (NAA) or N-acetylaspartate/creatine ratios (NAA/Cr) are the most common finding reported in subjects with AD and MCI [37-39], although alterations in other metabolites including myo-inositol [40] and glutamate [41] are also found. Glutamate levels have been studied on fewer occasions in MCI and AD. We previously demonstrated that glutamate levels are lower in AD and MCI than in healthy controls in the posterior cingulate gyrus [36, 42].

Additional longitudinal studies showed valuable results with MRS. In a large cohort of 151 MCI patients (most of them being of amnestic type) followed-up for 3 years, MRS was individually predictive of conversion to dementia but the accuracy of prediction improved when magnetic resonance spectroscopy was used in combination with hippocampal volumetry and the presence of cortical infarctions [43]. The value of proton magnetic resonance spectroscopy as a biomarker was assessed ante-mortem in a single study with 54 patients ranging from low to high likelihood of having AD and who underwent autopsy. Decreases in N-acetylaspartate/creatine and increases in myo-inositol/creatine ratios in the posterior bilateral cingulate gyrus correlated with higher postmortem Braak neurofibrillary tangle staging [16].

There are some caveats that influence metabolite measurements, such as magnetic field heterogeneity and cerebrospinal fluid (CSF), spatial resolution, signal-to-noise ratio, contrast-to-noise ratio contamination and artifacts. With the modern 3T scanners with smaller voxel analysed have overcome these limitations. Pitfalls in MRS can be minimised using automated and standard protocols. Given that the spectral patterns are well known, minor artifacts are relatively easy to identify.

With regard to other neuroradiological and biological predictors, SPECT has shown to be inferior to MRI techniques. According to the results of the largest ever cohort the overal accuracy was only 58%[44]. FDG-PET and amyloid-binding radiotracer PET are of superior value but both are costly and not widely available[38]. Protein tau and Beta42 amyloid peptide levels in the CSF are excellent predictors of conversion to AD[45] but they need lumbar puncture. APOE genotype is also useful but only in terms of specificity[15,38]. Therefore there is no clear consensus on what is the best biomarker of early AD.

In conclusion, both 1HMRS and brain volumetry are markers separately of cognitive decline and transition from MCI to AD. Given the high prevalence of AD and the predictive values below 80%, these techniques ar far from being useful as routine procedures in the clinical setting.

REFERENCES

1. Petersen RC, Knoopman D, Boeve BF, et al Mild cognitive impairment. Ten years later. Arch Neurol 2009; 66: 1447-1455.
2. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol 1991; 82: 239-259.
3. Esiri MM. An effective immune intervention for Alzheimer's disease in prospect? Trends Pharmacol Sci 2001; 22: 2-3.
4. Jack CR Jr, Petersen RC, Xu Y, et al. Rates of hippocampal atrophy correlate with change in clinical status in aging and AD. Neurology 2000; 55: 484-489.
5. Apostolova LG, Dutton RA, Dinov ID, et al. Conversion of mild cognitive impairment to Alzheimer disease predicted by hippocampal atrophy maps. Arch Neurol 2006; 63: 693-699.

6. Pennanen C, Kivipelto M, Tuomainen S, et al. Hippocampus and entorhinal cortex in mild cognitive impairment and early AD. Neurobiol Aging 2004; 25: 303-310.
7. DeCarli C, Frisoni GB, Clark CM, et al, Alzheimer's Disease Cooperative Study G. Qualitative estimates of medial temporal atrophy as a predictor of progression from mild cognitive impairment to dementia. Arch Neurol 2007; 64: 108-115.
8. Risacher SL, Saykin AJ, West JD, et al. Baseline MRI predictors of conversion from MCI to probable AD in the ADNI cohort. Curr Alzheimer Res 2009; 6: 347-361.
9. Jack CR Jr, Shiung MM, Gunter JL, et al. Comparison of different MRI brain atrophy rate measures with clinical disease progression in AD. Neurology 2004; 62: 591-600.
10. Nho K, Kim S, et al. Automatic Prediction of Conversion from Mild Cognitive Impairment to Probable AAMIA Annu Symp Proc.lzheimer's Disease using Structural Magnetic Resonance Imaging. 2010; 13:542-6.
11. Peters F, Villeneuve S, Belleville S. Predicting progression to dementia in elderly subjects with mild cognitive impairment using both cognitive and neuroimaging predictors. J Alzheimers Dis 2014;38:307-318.
12. Duchesne S, Bocti C, De Sousa K, et al. Amnestic MCI future clinical status prediction using baseline MRI features. Neurobiol Aging 2010; 31: 1606-1617.
13. Modrego PJ, Fayed N, Pina MA, Conversion from mild cognitive impairment to probable Alzheimer's disease predicted by brain magnetic resonance spectroscopy. American Journal of Psychiatry 2005;162:667-75.
14. Fayed N, Dávila J Oliveros A, et al. Utility of different MR modalities in mild cognitive impairment and its use as a predictor of conversion to probable dementia. Academic Radiology 2008; 15:1089-98.
15. Modrego PJ, Fayed N, Sarasa M, Magnetic resonance spectroscopy in the prediction of early conversion from amnestic mild cognitive impairment to dementia: a prospective cohort study. BMJ Open 2011; 1(1):e000007.
16. Kantarci K, Knopman DS, Dickson DW, et al. Alzheimer disease: Postmortem neuropathologic correlates of antemortem 1H MR spectroscopy metabolite measurements. Radiology 2008; 248: 210-220.
17. McKhan G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group. Neurology. 1984;34: 939-944.
18. Buschke H, Kuslansky G, Katz M, et al. Screening for dementia with the memory impairment screen. Neurology 1999;52:231-8.
19. Lobo A, Ezquerra J, Gomez Burgada F, et al. El Mini-Examen cognoscitivo: un test sencillo y práctico para detectar alteraciones intelectuales en pacientes médicos (in Spanish). Actas Luso Esp Neurol Psiquiatr Cienc Afines 1979;7:189-202.
20. Provencher SW. Estimation of metabolite concentrations from localised in vivo proton NMR spectra. Magn Reson Med 1993; 30:672–679.
21. Fayed N, Modrego PJ, Medrano J. Comparative test-retest reliability of metabolite values assessed with magnetic resonance spectroscopy of the brain. The LCModel versus the manufacturer software. Neurol Res 2009;31:472e7.
22. Modrego PJ, Fayed N, Errea JM, et al. Memantine versus donepezil in Alzheimer's disease. A randomized trial with magnetic resonance spectroscopy. Eur J Neurol 2010;17:405e12.
23. Manjón JV, Carbonell-Caballero J, Lull JJ, et al. MRI denoising using non-local means. Med Image Anal. 2008;12:514-23.

24. Manjón JV, Lull JJ, Carbonell-Caballero J, et al. A nonparametric MRI inhomogeneity correction method. Med Image Anal. 2007;11:336-45.
25. Kantarci K, Weigand SD, Przybelski SA, et al. MRI and MRS predictors of mild cognitive impairment in a population based sample. Neurology 2013;**81**:118-133.
26. Modrego PJ, Fayed N, Longitudinal magnetic resonance spectroscopy as marker of cognitive deterioration in mild cognitive impairment. Am J Alzheimers Dis Other Demen 2011; 26: 631-636.
27. Westman E, Wahlund LO, Foy C, et al. Magnetic resonance imaging and magnetic resonance spectroscopy for detection of early Alzheimer's disease. J Alzheimers Dis 2011;26:307-319.
28. , Hata S, Suzuki T, et al. Utility of SPM8 plus DARTEL (VSRAD) combined with magnetic resonance spectroscopy as adjunct techniques for screening and predicting dementia due to Alzheimer's disease in clinical practice. J Alzheimers Dis. 2014;41:1207-22.
29. Boccardi M, Ganzola R, Bocchetta M, et al. Survey of protocols for the manual segmentation of the hippocampus: preparatory steps towards a joint EADC-ADNI Harmonized Protocol. J Alzheimers Dis 2011;26:61-75.
30. McEvoy L, Fennema-Notestine C, Cooper Roddey J, et al. For the Alzheimer's Disease Neuroimaging Initiative Alzheimer Disease: Quantitative structural neuroimaging for detection and prediction of clinical and structural changes in mild cognitive impairment. Radiology 2009;251:195-205.
31. Jack C, Petersen R, Xu Y, et al. Medial temporal atrophy on MRI in normal aging and very mild Alzheimer's disease. Neurology 1997;49:786-794.
32. Erten-Lyons D, Howieson D, Moore MM, et al. Brain volume loss in MCI predicts dementia. Waragai MNeurology 2006;66:233-235.
33. Ioannidis JP, Excess significance bias in the literature on brain volume abnormalities. Arch Gen Psychiatry 2011;68:773-780.
34. Kantarci K, Proton MRS in mild cognitive impairment. J Magn Reson Imaging2013; 37:770-777.
35. Kantarci K, Boeve BF, Wszolek ZK, et al. MRS in presymptomatic MAPT mutation carriers: A potential biomarker for taumediated pathology. Neurology 2010;75:771-778.
36. Fayed N, Andrés E, Viguera L, et al. Higher glutamate+glutamine and reduction of N-acetyl-aspartate in posterior cingulate according to age range in patients with cognitive impairment and/or pain. Academic Radiology 2014;21:1211-1217.
37. Rapoport SI, Hydrogen magnetic resonance spectroscopy in Alzheimer's disease. Lancet Neurol 2002;1:82.
38. Modrego PJ, Predictors of conversion to dementia of probable Alzheimer type in patients with mild cognitive impairment. Curr Alzheimer Res 2006;3:161-170.
39. Valenzuela MJ, Sachdev P, Magnetic resonance spectroscopy in AD. Neurology 2001;56:592-598.
40. Siger M, Schuff N, Z hu X, et al. Regional myo-inositol concentration in mild cognitive impairment using 1H magnetic resonance spectroscopic imaging. Alzheimer Dis Assoc Disord 2009;23:57-62.
41. Rupsingh R, Borrie M, Smith M, et al. Reduced hippocampal glutamate in Alzheimer disease. Neurobiol Aging 2011;32:802-810.
42. Fayed N, Modrego PJ, Rojas-Salinas G, et al. Brain glutamate levels are decreased in Alzheimer's disease. A Magnetic Resonance Spectroscopy study. Am J Alzheimers Dis Other Demen 2011;26:450-456.

43. Kantarci K, Weigand SD, Przybelski SA, et al. Risk of dementia in MCI. Combined effect of cerebrovascular disease, volumetric MRI, and H MRS. Neurology 2009;72:1519-1525.
44. Ito K, Mori E, Fukuyama H, et al. Prediction of outcomes in MCI with (123)I-IMP-CBF SPECT: a multi-center prospective cohort study. Ann Nucl Med 2013; 27: 898-906
45. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol 2016; 15: 673-684

Table 1. Demographic variables and scales scores in the cohort of 48 patients with mild cognitive impairment

	Mean SD	Significance
Age, years Female Male	 73.7 (7.1) 75.2 (6.7)	F=0.51; P=0.476
Gender (female/male)	30/18	
Gender and volumetry: GM and WM		F=28.14 ; P<0.001 F=29.79; P=<0.001
Age and GM		r= -0.40; P=0.005
Convert/Not convert	9/21 6/12	F=8.34; P=0.006
MEC	28.3 (3.1)	
Blessed Dementia Rating Scale	2.6 (0.9)	
Memory Impairment Screen	2.3 (1.6)	

Rey Auditory Verbal Learning test	3.2 (2.6)	
Mean follow-up months	36 (6.7)	

Table 2. a) ANCOVA performed on morphometric variables (normalized by Intracranial Volume Total values). Significant variables.

Localization	**F**	**P**
Third ventricle	4.382	0.042

Table 2. b) ANCOVA on spectral data. Significant variables.

Localization and metabolites	**F**	**P**
Occipital Myo-inositol	6.332	0.016
Post Cingulate Glu/Cr	4.178	0.047
Post Cingulate Glx	4.128	0.049

Glu: glutamate; Cr: creatine; Glx: glutamate+glutamine; Post: posterior

TABLE 3. Relative volumes (in %):

	Group	**Mean**	**Standard Deviation**
Total Gray Matter	Non-converters	53,383	2,976
	Converters	52,593	2,373

Total White Matter	Non-converters	43,330	2,650
	Converters	42,582	2,052
Lateral Ventricle Right	Non-converters	1,422	0,762
	Converters	2,074	0,965
Lateral Ventricle Left	Non-converters	1,523	0,784
	Converters	2,335	0,824
Fourth Ventricle	Non-converters	0,151	0,067
	Converters	0,167	0,058
Third Ventricle	Non-converters	0,192	0,055
	Converters	0,249	0,053
Frontal Lobe Right	Non-converters	6,426	0,592
	Converters	6,315	0,317
Frontal Lobe Left	Non-converters	6,336	0,536
	Converters	6,186	0,468
Temporal Lobe Right	Non-converters	4,279	0,319
	Converters	4,208	0,294
Temporal Lobe Left	Non-converters	4,262	0,340
	Converters	4,111	0,350
Parietal Lobe Right	Non-converters	4,640	0,311
	Converters	4,440	0,343
Parietal Lobe Left	Non-converters	4,480	0,354
	Converters	4,340	0,407
Occipital Lobe Right	Non-converters	2,017	0,212
	Converters	1,970	0,142
Occipital Lobe Left	Non-converters	1,997	0,234
	Converters	1,914	0,214
Cingulated Gyrus Right	Non-converters	0,788	0,116
	Converters	0,753	0,098
Cingulated Gyrus Left	Non-converters	0,802	0,131
	Converters	0,770	0,070

Thalamus Right	Non-converters	0,614	0,068
	Converters	0,606	0,056
Thalamus Left	Non-converters	0,664	0,065
	Converters	0,663	0,073
Caudate Nucleus Right	Non-converters	0,334	0,046
	Converters	0,351	0,059
Caudate Nucleus Left	Non-converters	0,329	0,041
	Converters	0,337	0,057
Putamen Right	Non-converters	0,442	0,067
	Converters	0,446	0,056
Putamen Left	Non-converters	0,477	0,055
	Converters	0,486	0,067
Globus Pallidus Right	Non-converters	0,144	0,021
	Converters	0,147	0,023
Globus Pallidus Left	Non-converters	0,146	0,018
	Converters	0,155	0,020
Hippocampus Right	Non-converters	0,377	0,053
	Converters	0,351	0,075
Hippocampus Left	Non-converters	0,364	0,059
	Converters	0,324	0,060
Amygdala Right	Non-converters	0,141	0,025
	Converters	0,137	0,025
Amygdala Left	Non-converters	0,134	0,027
	Converters	0,121	0,023
Cerebellum Gray Matter Right	Non-converters	5,103	0,459
	Converters	5,293	0,513
Cerebellum Gray Matter Left	Non-converters	4,999	0,442
	Converters	5,176	0,404
Cerebellum White	Non-converters	1,390	0,218

Matter Right			
	Converters	1,394	0,212
Cerebellum White Matter Left	Non-converters	1,332	0,178
	Converters	1,302	0,173

Figure 1. Voxel placement

1a. Sagittal and occipital T1-weighted magnetic resonance imaging with the voxel placed in the ventral posterior cingulate gyrus and inferior precuneus (a) and occipital cortex (b)

Figure 1b Example of spectrum with the following peaks: mI, myo-inositol; Cho, choline compounds; Cr, creatine; Glx, glutamate + glutamine + GABA; NAA, N-acetylaspartate; ppm, parts per million (c,d).

Figure 2. Baseline brain morphometry and spectroscopy as a predictor of conversion from mild cognitive impairment to probable Alzheimer's disease. ROC curves.

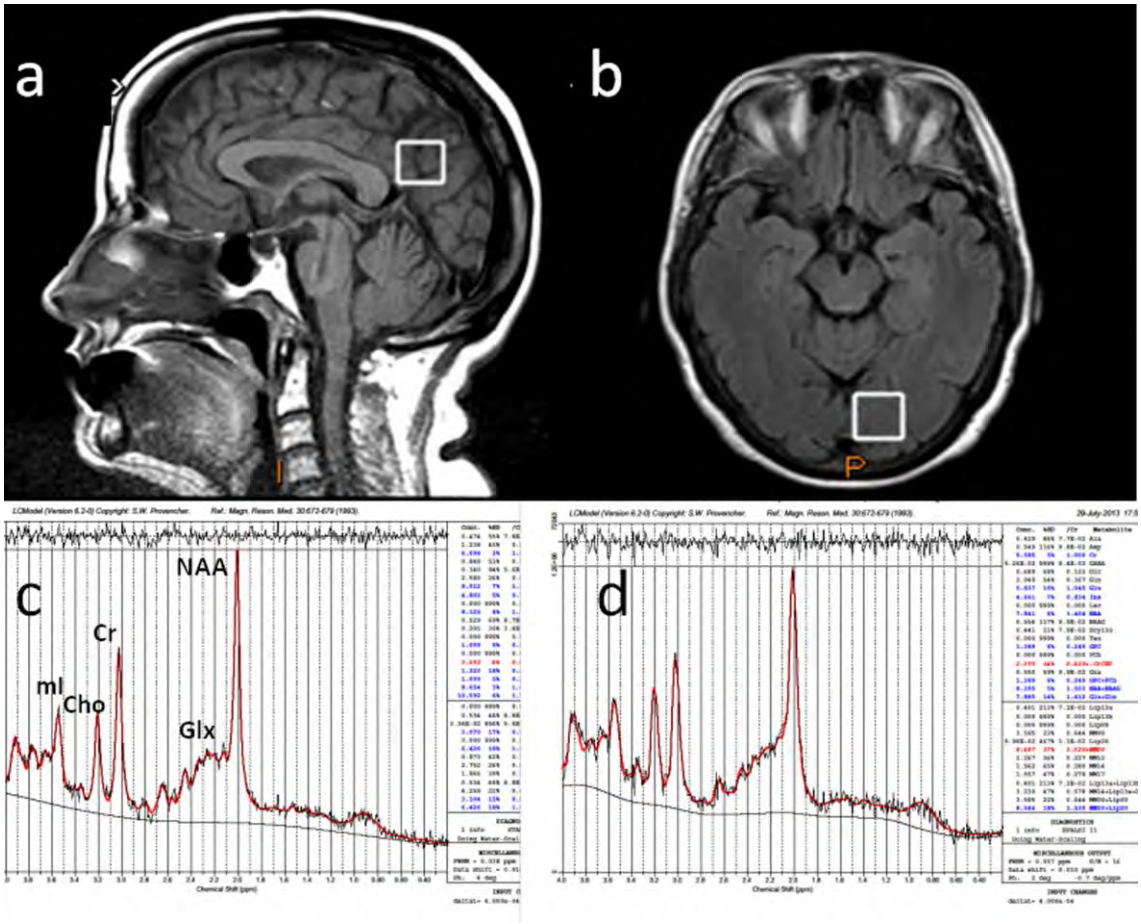

Figure 1a

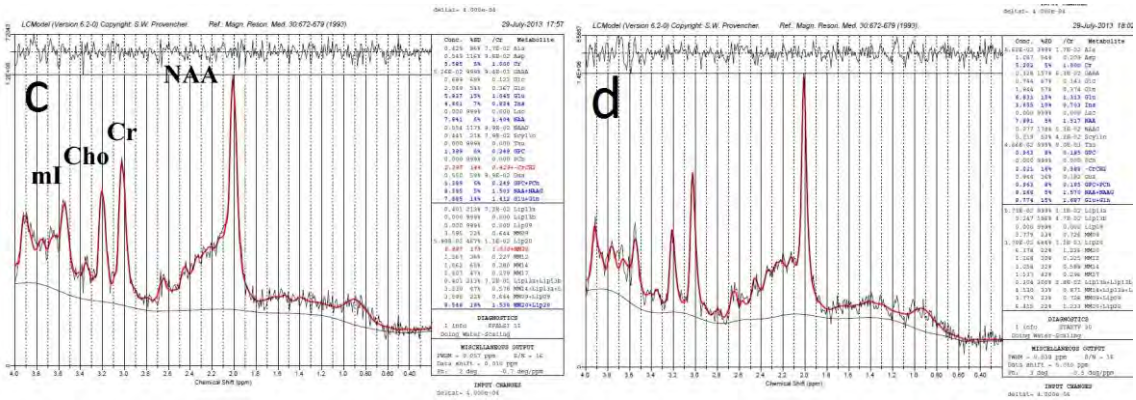

Figure 1b

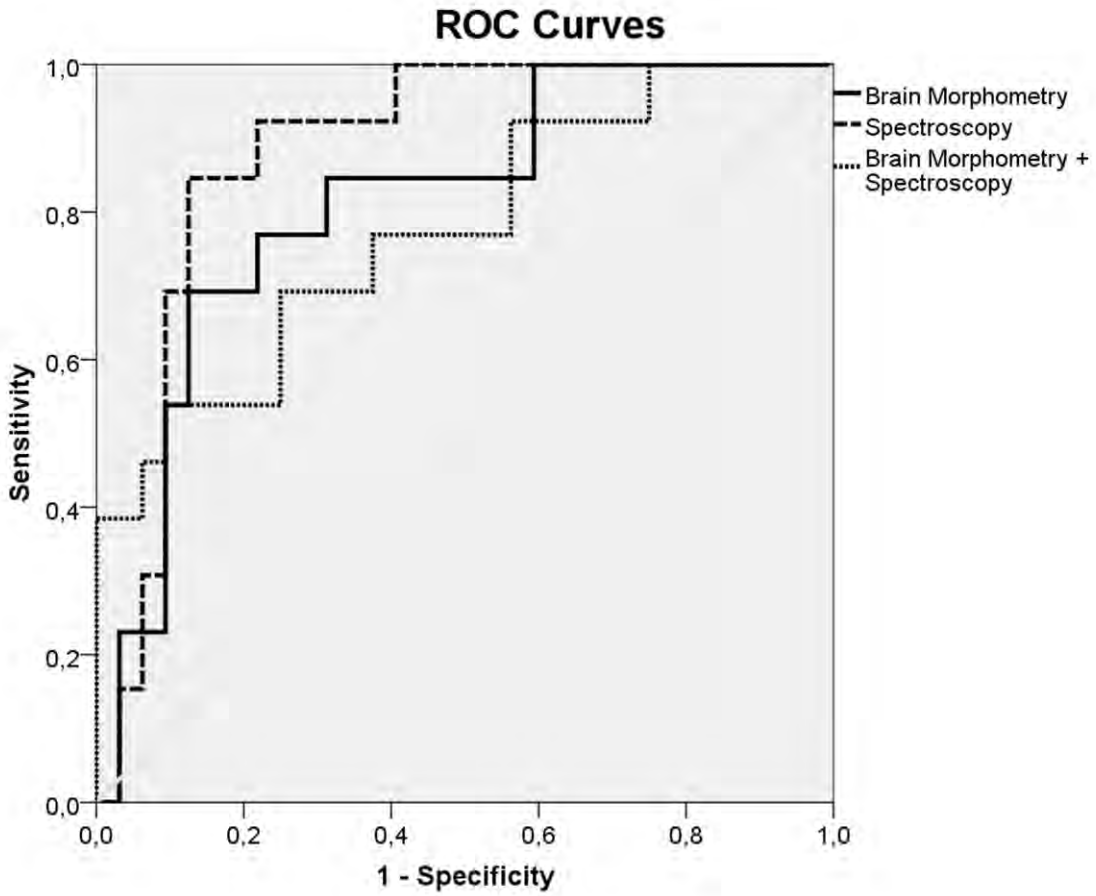
ROC Curves
Sensitivity
1 - Specificity
0,0
0,2
0,4
0,6
0,8
1,0
Brain Morphometry
Spectroscopy
Brain Morphometry + Spectroscopy

Figure 2