Previous versions

Version 1: BMJ Open-2010-000007

Title: Magnetic Resonance Spectroscopy in the prediction of early conversion from amnestic Mild Cognitive Impairment to dementia.

Authors: Pedro J Modrego[a], Nicolas Fayed,[b] Manuel Sarasa[c]

a) Department of Neurology. Hospital Universitario Miguel Servet. Zaragoza. Spain
b) Department of Radiology. Clinica Quiron. Zaragoza. Spain
c) Laboratory of Neurobiology. University of Zaragoza. Spain

Correspondence:

Dr PJ Modrego

Department of Neurology

Hospital Miguel Servet

50009 Zaragoza

Spain

e-mail: pmodrego@salud.aragon.es

ABSTRACT

Background and objective. Mild Cognitive Impairment (MCI) of amnestic type is a common condition in the elderly highly predictive of Alzheimer's disease (AD). To date there is no clear consensus on what is the best tool to predict early conversion.

To demonstrate that ^{1}H Magnetic Resonance Spectroscopy (MRS) of the brain may predict early conversion to dementia within the 2 year period after baseline assessment.

Methods. A cohort of patients fulfilling the criteria of amnestic MCI were enrolled consecutively. At baseline we carried out neuropsychological examination, Standard Blood tests, and APOE genotype. 1.5T ^{1}H-Magnetic Resonance Spectroscopy of the brain was carried out by exploring two areas: posteromedial bilateral parietal lobe and left medial occipital lobe.

The patients were followed-up to detect conversion to probable Alzheimer's disease according to the NINCDS-DRDA group criteria.

RESULTS. After 2 year mean follow-up 27 (38%) patients converted to AD. The mean NAA/Cr ratio in the posteromedial bilateral parietal cortex was 1.38 in converters versus 1.49 in non-converters ($p<0.0001$). A NAA/Cr ratio equal to or lower than 1.43 in this area predicted conversion to probable AD at 74.1% sensitivity and 83.7% specificity (area under the curve: 0.84; 95% CI: 0.73-0.92). The cross-validated accuracy of classification was 82%, which reaches an 85% when APOE4 genotype and memory test are included in the analysis. In the left medial occipital lobe the predictive value was somewhat lower with 85.2% sensitivity and 61.4% specificity (area under the curve: 0.8; 95% CI: 0.69-0.89).

CONCLUSION. MRS is a useful tool to predict early conversion to dementia in patients with amnestic MCI.

Key words: Mild Cognitive Impairment. Magnetic Resonance Spectroscopy.

What it is already known

Amnestic mild cognitive impairment is a common condition at increased risk of conversion to Alzheimer's disease. There is no clear consensus on what is the best biomarker to predict early conversion

There are a few longitudinal studies using brain Magnetic Resonance Spectroscopy as predictor of conversion t dementia.

What this study adds.

Magnetic Resonance Spectroscopy is a reliable biomarker of AD and predicts conversion to dementia. T

The posteromedial parietal cortex being the best area to be explored in terms of accuracy of classification.

Mild cognitive impairment (MCI) is a common condition in the elderly mainly characterized by memory loss. Although there may be other subtle inefficiencies however the general cognitive function and daily living activities are preserved[1]. The meaning of this concept varies across the scientific community: a transitional state between normality and dementia[1], an early phase of Alzheimer's disease (AD)[2], or an unstable condition that may evolve to dementia or may even revert to normality[3]. Regardless of conceptualization most patients convert to AD over time but some of them remain non-demented. Therefore we need a biomarker to predict conversion to dementia to start treatment as soon as possible. Many works focused on biomarkers of AD to diagnose this disease in the early phase called MCI or even before. Apart from the genetic mutations in familial cases there is no marker accurate enough for AD. It is true that the atrophy of the medial temporal lobe structures, such as entorhinal cortex and hippocampus, has reached encouraging results[4-12], and CSF biomarkers (tau and Abeta42 proteins) as well[13-19], but no standardized procedures have been established and there is a large intra and inter-individual variations.

In the search for more objective and reproducible markers Magnetic Resonance Spectroscopy (MRS) of the brain was used for prediction purposes and found that the hippocampal levels of N-acetyl-aspartate (NAA/creatine ratios) were not predictive of conversion to dementia but the values in the left occipital lobe did so with excellent sensitivity but with a specificity of 75%.[20] Given that this longitudinal study was conducted in 53 MCI patients only it is necessary to confirm the findings in larger cohorts and to investigate other areas of the brain. Studies with PET in MCI and AD show the areas involved in early phases. The posterior cingulate gyrus (PCG) is an area involved in memory and many times studied in MCI and AD. The patients with AD had lower glucose metabolism than healthy controls in parietal, temporal, occipital, frontal and posterior cingulate cortices.[21] FDDNP-PET (radiotracer binding to plaques and tangles)[22] and FDG-PET[23] findings can discriminate normality from MCI and AD with the PCG being one of the most typically affected areas.

Several cross-sectional MRS studies found decreased NAA/Cr levels in the PCG of AD patients in comparison with controls,[24-26] and in the occipital lobe as well.[27-29] Another cross-sectional study including 24 MCI patients and 22 with AD showed that the findings in the posterior cingulate gyrus (NAA/Cr ratios) discriminated both conditions at 67% sensitivity and 80% specificity.[30] In another report of the same authors the myo-inositol/Cr ratios in the PCG were higher in AD and MCI patients than in controls.[25]

A previous study of 119 MCI patients compared the NAA/Cr ratios in the left occipital lobe with those obtained in the posteromedial parietal cortex (PMPC) yielding similar predictive values, with the PMPC values being a little more valuable than those observed in the occipital lobe. This study included amnestic and multiple domain MCI. At baseline it was observed a significant correlation between the ratios observed in these two locations.[31]

On the basis of all of the above and, given the paucity of longitudinal MRS studies in MCI, the purpose of this work is to investigate whether MRS measuring of the cerebral NAA/Cr ratios in amnestic MCI are predictive of early conversion to dementia. We hypothesize that the occipital and parietal values are similarly predictive.

PATIENTS AND METHODS

A cohort of patients fulfilling the criteria of amnestic MCI according to the Petersen et al[1] were recruited consecutively. The patients were referred by family physicians, and in all cases an informant must corroborate the memory problems. At baseline the patients underwent neuropsychological analysis encompassing Mini-Mental test (Spanish version with a maximum

possible score of 35 points)[32], Memory Impairment Screen (MIS)[33], Blessed Dementia Rating Scale, clock drawing test, GDS (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 normal score in the Mini-Mental. The cut-off points for the RAVLT 20 minute delayed recall were as follows: =/< 4 for patients aged up to 69, and =/<3 for the patient aged 70 and older. Those who scored 11 points or higher in the GDS were re-evaluated after antidepressant treatment so as to confirm that they had MCI.

The patients fulfilling the criteria mentioned above underwent also standard blood tests including B12 vitamin, serologic test of syphilis, and thyroid hormones. APOE genotype was also carried out. Brain Magnetic Resonance techniques were also carried out as follows. All patients underwent brain T1and T2-weighted MRI on a 1.5 T clinical scanner (Signa HD, GE). Single-voxel ^{1}H-MRS was carried out by means of an echo time (TE) of 35 milliseconds and a repetition time (TR) of 2000 milliseconds with spin echo technique that uses selective excitation with gradient spoiling for 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; 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 GE software (Signa HD, GE software release 12.x), version 3.0, 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 volume voxel was 2x2x2 cm in one of every area explored. These areas of exploration were the left medial occipital lobe and the posteromedial parietal area bilaterally encompassing the posterior cingulated gyrus and the inferior precuneus (see figures 1 and 2). Spectra were rejected and repeated in the following cases: linewidth >10 Hz, lineshape asymmetric after eddy current correction, and the presence of artifacts. Data fits with %SD>20 from the Cramer-Rao inequality were eliminated.

Both areas we examined showed excellent reproducibility in two previous studies of test-retest reliability done with the same clinical scanner in AD patients.[34,35] For the NAA/Cr ratios the alpha 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. In spite of these good alpha values seen previously we also carried out a second immediate MRS in 22 patients without being removed from the scanner to check reproducibility. The intra-class correlation coefficients were also remarkable (0.92 and 0.9 for parietal and occipital areas respectively).

At baseline we also carried out MRS in 35 healthy elderly controls with the voxels located in the same areas for comparison purposes.

The patients were followed-up and re-evaluated every 6 months or earlier to check if they converted to dementia of probable AD type according to the NINCDS-ADRDA group criteria.[36] Re-assessment was based on Mini-mental, MIS, BDRS, and clock drawing test.

Statistical analysis

The comparison of quantitative variables such as metabolite values in converters and non-converters was made with two-tailed t-tests. The predictive values of the different variables (APOE4, memory tests, and brain metabolite values) were calculated with the analysis of ROC curves and discriminant analysis. Parameters such as sensitivity, specificity, positive and negative predictive values, and accuracy of classifications are reported. The results were cross-validated. ROC curves were analyzed with Med-Calc software, and discriminant analysis with the SPSS, version 10.

We obtained informed consent from patients and relatives. This study was approved by our regional ethical committee.

RESULTS

Initially we recruited a cohort of 78 patients scoring 5 or lower in the MIS and that fulfilled the criteria of amnestic MCI. However MRS was not possible in 6 cases; 3 had claustrophobia, two bore pacemaker, and one refused participation. One patient had to be excluded because an incidental brain-stem tumor. Therefore 71 patients were finally included in the study. In table 1 are reported the main baseline demographic variables and the results of memory tests and scales. After a mean follow-up of 2 years (range: 1.5-2.5 years), 27 (38%) patients out of 71 converted to probable AD according to NINCDS-ADRDA criteria, and none of them reverted to normal.

Spectroscopic results.

Controls versus MCI patients

In table 2 are reported the values of the different variables for converters and non-converters, and also for controls. When we compared the NAA/Cr ratios in the occipital lobe of the 71 MCI patients with 35 controls we saw significant differences. It was 1.56 (SD: 0.09) in patients in comparison with 1.65 (SD: 0.08) in controls (t= 5; p<0.0001). No significant differences were seen in the rest of metabolite ratios. In the posteromedial parietal cortex no global differences were observed in the mean NAA/Cr ratios which was 1,46 for controls and 1,45 for MCI patients. The differences in the NAA levels were significant: 134.06 (SD: 18.3) for controls versus 123.17 (SD: 17,3) for MCI patients (t=2.99; p=0.003). We did not see significant differences for the other metabolites and ratios.

Converters versus non-converters.

The mean NAA/Cr ratio in the posteromedial cortex was 1.30 (SD:0.09) in converters versus 1.49 (SD: 0.08) in non-converters (t=9.96; p<0.0001). In the occipital lobe it was 1.48 (SD: 0.08) in converters versus 1.6 in non-converters (t=4, 89; p=0.0001). The absolute occipital NAA level was 133.7 (SD: 25.1) in converters versus 146.9 (SD: 24.4) in non converters (p=0.03). The differences were not significant for the other metabolite values (see table 2). It is worth mentioning that the baseline NAA/Cr values in the posteromedial and occipital cortices correlated significantly (r=0.56; p<0.0001) in the whole sample of 71 patients.

A NAA/Cr ratio equal to or lower than 1.43 in the posteromedial bilateral parietal cortex predicted conversion to probable AD at 74.1% sensitivity and 83.7% specificity (area under the curve: 0.84; 95% CI: 0.73-0.92). The cross-validated accuracy of classification was 82%, which reaches an 85% when APOE genotype and memory test are included in the analysis. In the left medial occipital lobe the predictive value was somewhat lower with 85.2% sensitivity and 61.4% specificity (area under the curve: 0.8; 95% CI: 0.69-0.89). The ROC curves are presented in figure 3; the dot plots representing the values of NAA/Cr ratios in parietal and occipital cortices are depicted in figures 4 and 5 respectively. Although the differences were highly significant there was some degree of overlapping in the NAA/Cr ratios as it is presented in these figures.

The APOE4 genotype alone yielded low predictive values in terms of sensitivity as 18 patients converted to dementia in spite of not having APOE4 alleles and only 8 of converters had one or two alleles (33% sensitivity and 72% specificity). The RAVLT yielded low sensitivity but high specificity (55.6% and 84% respectively).

.

DISCUSSION

The diagnosis of AD in early phases is still challenging. Several tools, clinical and radiological, have been used so far but there is not a consensus on what is the best. In addition determined techniques are not available in every medical center, so each department should take advantage of the available ones. Volumetry of the medial temporal lobe structures is widely used but not free of limitations (artifacts, lack of standardization, complexity and long duration of the process). At a fixed 80% specificity the hippocampus plus entorhinal cortex volume predicted conversion to AD at 66.7% sensitivity in a cohort of 139 MCI patients followed-up for 5 years; when other variables such as age and scores in neuropsychological scores were added then sensitivity jumped to 83.3% with 86.8% of patients correctly classified.[37]

According to a large prospective prospective multicenter cohort results with 750 MCI subjects followed-up for a minimum of 2 years, the combination of Aβ42/P-tau ratio and T-tau in the CSF predicted conversion to AD with a sensitivity of 83%, a specificity of 72%, a positive predictive value of 62% and a negative predictive value of 88%, but the analytical techniques are pending standardization.[38] Combining hippocampal volumetry and CSF biomarkers outperformed the individual use of either technique in the Goteborg MCI study with 90% accuracy of prediction.[39] To date the best accuracy to predict early conversion to AD has been obtained with FDG-PET, with both sensitivities and specificities higher than 80%, and even higher than 90% when combined with APOE4 genotype or memory tests in follow-up periods hardly longer than one year.[40-43] The excellent values found in the previous studies have not been confirmed in the cohort of the AD Neuroimaging Initiative group (ADNI) where the positive predictive valued was 41% and the negative one was 79%.[44]

PET with Pittsburg radiotracer (amyloid plaques binding) also seems promising in terms of specificity. In a study including 31 MCI patients as 14 (82% of PIB positive patients) converted to dementia over 3 years. However, only 17 MCI patients (55%) were PIB positive at baseline.[45]

In general neuropsycholgical tests alone barely surpass 75% accuracy of prediction according to an extensive review.[46] However two studies disclosed an accuracy of prediction of around 90%.[47,48] More recently a study including 148 patients with MCI revealed that the combination of two measures (delayed recall on the Selective Reminding Test, and Digit Symbol test from the WAIS) yielded an accuracy level of 86%.[49]

MRS represents a valuable technique in AD on the basis of previous published works. It was found that the NAA was lower in the brain of AD than in controls and that this decrease correlated

with the number of neuritic plaques and neurofibrillary tangles in tissue sections.[50] The value of proton MRS as a biomarker has been assessed ante-mortem in a series of 54 patients ranging from low to high likelihood of having AD and who underwent autopsy. Decreases in NAA/Cr and increases in mI/Cr ratios correlated with higher Braak neuropathological stages in the posterior bilateral cingulate gyrus.[51] In a small cohort of MCI (15) patients and controls (12) the ratios of NAA/Cr in the parietal lobe decreased longitudinally more in patients who converted to dementia than in non-converters. [52] Another small MRS study (25 MCI patients) with shorter follow-up (one year) showed significant differences in the NAA/ Cr ratio of the left paratrigonal white matter between converters and non-converters.[53]

Although the hippocampus is theoretically a good area to explore in MCI and early AD, some drawbacks should be mentioned: the excessive volume of the voxel analyzed (8 cc) may include extrahippocampal tissues, the possible artifacts in the magnetic field, and the proximity of the hippocampus to osseous structures or air cavities makes this area difficult to explore with MRS. It is likely that modern 3T scanners with smaller voxel analyzed will overcome these limitations.[54,55]

In conclusion MRS is a useful technique as biomarker in early Alzheimer's disease as it predicts early conversion to dementia. Although perhaps inferior to FDG-PET, it could yield similar performance to structural neuroimaging and CSF biomarkers. We think MRS may play a role where no better techniques are available.

ACKNOWLEDGEMENTS

This study has been conducted with the financial support of the Spanish Ministry of Education and Science (SAF 2006-13332). None of the authors has conflicts of interest to declare.

AUTHORS CONTRIBUTION

Dr PJ Modrego: design, clinical data acquisition. Statistical analysis. Drafting of the manuscript.

Dr N Fayed: Magnetic Resonance Imaging acquisition. Design. Critical review.

Dr M Sarasa: acquisition of funding. APOE genotype determination. Critical review.

GUARANTOR OF THE STUDY: Dr PJ Modrego

REFERENCES

1. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild Cognitive Impairment. Clinical characterization and outcome. Arch Neurol. 1999; 56: 303-308.

2. Morris JC, Storandt M, Miller JP, et al. Mild Cognitive Impairment represents early-stage Alzheimer's disease. Arch Neurol. 2001; 58: 397-405.

3. Larrieu S, Letenneur L, Orgogozo JM, et al. Incidence and outcome of mild cognitive impairment in a population based cohort. Neurology. 2002; 59: 1594-1599.

4. De León MJ, Golomb J, George AE, et al. The radiologic prediction of Alzheimer's disease: The atrophic hippocampal formation. Am J Neuroradiol. 1993; 14; 897-906.

5. Jack CR, Petersen RC, Xu YC, et al. Prediction of AD with MRI based hippocampal volume in mild cognitive impairment. Neurology 1999; 52: 1397-1403

6. Killiany RJ, Gómez-Isla T, Moss M et al. Use of structural magnetic resonance imaging to predict who will get Alzheimer's disease. Ann Neurol. 2000; 47: 430-439.

7-Dickerson BC, Goncharova I, Sullivan MP et al. MRI-derived entorhinal and hippocampal atrophy in incipient and very mild AD. Neurobiol Aging. 2001; 22: 747-754.

8-Visser PJ, Verhey FR, Hofman PA, Scheltens P, Jolles JP. Medial temporal lobe atrophy predicts Alzheimer's disease in mild cognitive impairment. J Neurol Neurosurg Psychiatry. 2002; 72: 491-497.

9. Korf ES, Wahlund LO, Visser PJ, Scheltens P. Medial temporal lobe on MRI predicts dementia in patients with mild cognitive impairment. Neurology. 2004; 63: 94-100.

10. De Toledo-Morrell L, Stoub TR, Bulgakova M, et al. MRI-derived entorhinal volume is a good predictor of conversion from MCI to AD. Neurobiol Aging. 2004; 25: 1197-1203.

11. Jack CR, Shiung MM, Weigand SD, et al. Brain atrophy rates predict subsequent clinical conversion in normal elderly with amnestic MCI. Neurology. 2005; 65: 1227-1231.

12. Stoub TR, Bulgakova M, Leurgans S, et al. MRI predictors of risk of incident Alzheimer's disease: a longitudinal study. Neurology. 2005; 64: 1520-1524.

13. Riemenschneider M, Lautenschlager N,Wagenpfeil S, Diehl J, Drzezga A, Kurz A. Cerebrospinal fluid tau and beta-amyloid 42 proteins identify Alzheimer's disease in subjects with mild cognitive impairment. Arch Neurol. 2002; 59: 1729-1734.

14. Zatterberg H, Wahlung LO, Blennow K. Cerebrospinal fluid markers for prediction of Alzheimer's disease. Neurosci Lett. 2003; 352-67-69.

15. Andreasen N, Vanmechelen E, Vanderstichele H, Davidsson P, Blennow K. Cerebrospinal fluid levels of total-tau, phospho-tau and A Beta42 predicts development of Alzheimer's disease in patients with mild cognitive impairment.Acta Neurol Scand. 2003; 179: 47-51.

16. Hampel H,Teipel SJ, Fuchrberger T, et al. Value of CSF-Beta-amyloid1-42 and tau as predictors of Alzheimer's disease in mild cognitive impairment. Mol Psychiatry. 2004; 9:705-710.

17. Ivanoiu A, Sindic CJ. Cerebrospinal fluid Tau protein and Amyloid-B42 in mild cognitive impairment: prediction of progression to Alzheimer's disease and correlation with the neurological examination. Neurocase. 2005; 11: 32-39.

18. de Leon MJ, Segal S, Tarshish CY, et al. Longitudinal cerebrospinal fluid tau load increases in mild cognitive impairment. Neurosci Lett. 2002; 333-183-186.

19. Vanderstichele H, De Meyer G, Andreasen N, et al. Amino-truncated {beta}-Amyloid42 peptides in cerebrospinal fluid and prediction of progression of mild cognitive impairment. Clin Chem. 2005; 51: 1650-1660.

20. Modrego PJ, Fayed N, Pina MA. Conversión from Mild Cognitive Impairment to probable Alzheimer's disease predicted by brain Magnetic Resonance Spectroscopy. Am J Psychiatry. 2005; 162: 667-675.

21. Alexander GE, Chen K, Pietrini P, Rapoport SI, Reiman EM. Longitudinal PET evaluation of cerebral metabolic decline in dementia : A potential outcome measure in Alzheimer's disease treatment studies. Am J Psychiatry. 2002; 159: 738-745.

22. Small GW, Kepe V, Ercoli LM, et al. PET of brain amyloid and tau in mild cognitive impairment. New Engl J Med. 2006; 355: 2652-2663.

23. Hunt A, Schonknecht P, Henze M, Seidl U, Haberkorn U, Schröder J. Reduced cerebral glucose metabolism in patients at risk for Alzheimer's disease. Psychiatry Res. 2007; 155: 147-154.

24. Kantarci K, Petersen RC, Boeve BF, et al. ^{1}H MR spectroscopy in common dementias. Neurology. 2004; 63: 1393-1398.

25. Kantarci K, Jack CR, Xu YC, et al. Regional metabolic patterns in mild cognitive impairment and Alzheimer's disease. Neurology. 2000; 55: 210-217.

26. Hancu I, Zimmerman EA, Sailasuta N, Hurd RE. H MR spectroscopy using TE averaged PRESS: A more sensitive technique to detect neurodegeneration associated with Alzheimer's disease. Magn Reson Med. 2005; 53: 777-782.

27. Miller BL, Moats RA, Shonk T, Earnst E, Wooley S, Ross BD. Alzheimer's disease: depiction of increased cerebral myoinositol with proton MR spectroscopy. Radiology. 1993; 187:433-437.

28. Shonk TK, Moats RA, Gifford P, Michaelis T, Mandigo JC, Izumi J, Ross BD. Probable Alzheimer Disease: diagnosis with Proton MR Spectroscopy. Radiology. 1995; 195: 65-72.

29. Rai GS, McConnel JR, Waldman A, Grant D, Chaudhry M. Brain proton spectroscopy in dementia: an aid to clinical diagnosis. Lancet. 1999; 353: 1063-1064.

30. Kantarci K, Xu YC, Shiung MM, et al. Comparative diagnostic utility of different MR modalities in Mild Cognitive Impairment and Alzheimer's disease. Dement Geriatr Cogn Disord. 2002; 14: 198-207.

31. Fayed N, Davila J, Oliveros A, castillo J, Medrano JJ. Utility of different MR modalities in mild cognitive impairment and its use as a predictor of conversión to probable dementia. Acad Radiol. 2008; 15: 1089-1098.

32. Lobo A, Ezquerra J, Gomez Burgada F, Sala JM, Seva Diaz A. El Mini-Examen cognoscitivo: un test sencillo y practico para detectar alteraciones intelectuales en pacientes médicos. Actas Luso Esp Neurol Psiquiatr Cienc Afines. 1979; 7:189-202.

33. Buschke H, Kuslansky G, Katz M, Stewart WF, Slwinski MJ, Eckholdt HM, Lipton RB. Screeing for dementia with the memory impairment screen. Neurology. 1999; 52: 231-238.

34. 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: 472-477.

35. Modrego PJ, Fayed N, Errea JM, Rios C, Pina MA, Sarasa M. Memantine versus donepezil in Alzheimer's disease. A randomized trial with Magnetic Resonance Spectroscopy. Eur J Neurol. 2010; 17: 405-412.

36. McKhan G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group. Neurology. 1984; 34: 939-944.

37. Devanand DP, Pradhaban G, Liu X, et al. Hippocampal and entorhinal atrophy in mild cognitive impairment : prediction of Alzheimer disease. Neurology. 2007; 68: 828-836.

38. Mattsson N, Zetterberg H, Hansson O, et al. CSF biomarkers and incipiente Alzheimer disease in patients with mild cognitive impairment. JAMA. 2009; 302: 385-393.

39. Eckerstrom C, Andreasson U, Olsson E, et al. Combination of hippocampal volume and cerebrospinal fluid biomarkers improves predictive value in mild cognitive impairment. Dement Geriatr Cogn Disord 2010; 29: 294-300

40. de Leon MJ, Convit A, Wolf OT, et al. Prediction of cognitive decline in normal elderly subjects with 2-[(18)F]fluoro-2-deoxy-D-glucose/positron-emission tomography (FDG/PET). Proc Natl Acad Sci USA. 2001; 98: 10966-10971.

41. Mosconi L, Perani D, Sorbi S, et al. MCI conversion to dementia and the APOE genotype: a prediction study with FDG-PET. Neurology 2004; 63: 2332-2340

42. Drzezga A, Grimmer T, Riemenschneider M, et al. Prediction of individual clinical outcome in MCI by means of genetic assessment and (18)F-FDG PET. J Nucl Med. 2005; 46: 1625-1632.

43. Anchisi D, Borroni B, Franceschi M, et al. Heterogeneity of brain glucose metabolism in mild cognitive impairment and clinical progression to Alzheimer's disease. Arch Neurol. 2005; 62: 1728-1733.

44. Landau SM, Harvey D,Madison CM, et al. Comparing predictors of conversion and decline in mild cognitive impairment. Neurology 2010; 75: 230-238

45. Okello A, Koivunen J, Edison P, et al. Conversión of amyloid positiveand negative MCI to AD over 3 years: an 11C-PIB PET study. Neurology. 2009; 73:754-760

46. Modrego PJ. Predictors of conversion to dementia of probable Alzheimer type in patients with mild cognitive impairment. Curr Alzh Res. 2006; 3: 161-170.

47. Kluger A, Ferris S[1]H, Golomb J, Mittelman MS, Reisberg B. Neuropsychological prediction of decline to dementia in nondemented elderly. J Geriatr Psychiatry Neurol. 1999; 12: 168-179.

48. Grober E, Lipton RB, Hall C, Crystal H. Memory impairment on free and cued selective reminding predicts dementia. Neurology. 2000; 54: 827-832.

49. Tabert MH, Manly JJ, Liu X, et al. Neuropsychological prediction of conversion to Alzheimer disease in patients with mild cognitive impairment. Arch Gen Psychiatry. 2006; 63: 916-924.

50. Klunk WE, Panchalingam K, Moossy J, McClure RJ, Pettegrew JW. N-acetyl-L-aspartate and other amino acid metabolites in Alzheime's disease brain: a preliminary proton nuclear magnetic resonance study. Neurology. 1992: 42: 1578-1585.

51. Kantarci K, Knopman DS, Dickson DW, et al. Alzheimer disease: Postmortem neuropathologic correlates of antemortem 1-H MR Spectroscopy metabolite measurements. Radiology. 2008; 248: 210-220.

52. Pilatus U, Lais C, Rochmont Adu M, et al. Conversion to dementia in mild cognitive impairment is associated with decline of N-acetylaspartate and creatine as revealed by magnetic resonance spectroscopy. Psychiatry Res. 2009; 173: 1-7.

53. Metastasio A, Rinaldi P, Tarducci R, et al. Conversión of MCI to dementia: Role of proton magnetic resonance spectroscopy. Neurobiol Aging. 2006; 27: 926-932.

54. Wang Z, Zhao C, Yu L, Zhou W, Li K. Reginal metabolic changes in the hippocampus and posterior cingulated area detected with 3-Tesla Magnetic resonance spectroscopy in pateients with mild cognitive impairment and Alzheimer's disease. Acta Radiol. 2009; 50: 312-319.

55. Caserta MT, Ragin A, Hermida AP, John Ahrens R, Wise L. Single voxel MR spectroscopy at 3T in a memory disorders clinic: early right hippocampal NAA/Cr loss in mildly impaired subjects. Psychiatry Res. 2008; 164: 154-159.

Variables (n=71)

AGE: 74.6 y (SD: 6.4; range: 58-88)
SEX: 43 were female
MEC: 28.4 (SD: 3.1; range: 21-34)
BDRS: 2.8 (SD: 0.9; range: 1-4)
MIS: 2.4 (SD: 1.7; range: 0-5)
RAVLT: 3.1 (SD: 2.5; range: 0-6) in delayed recall.

Elementary education: 65 patients
High school education: 6 patients
Higher education: 4 patients

Hypertension: 24 (33.8%)
APOE4 genotype: 21 had 1 or two alleles

Table 1. Demographic variables and scales scores in the cohort of 71 patients with amnestic MCI.

VARIABLE	Controls n=35	Converters N=27	Non-converters N=44	
Posteromedial parietal cortex				
NAA	134 (18.3)	120 (19.89	125 815.59	NS
NAA/Cr	1.46 (0.08)	**1.38 (0.09)**	**1.49 (0.08)**	**p<0.0001**
Ch/Cr	0.61 (0.07)	0.62 (0.05)	0.59 (0.1)	NS
mI/Cr	0.66 (0.08)	0.63 (0.08)	0.6 (0.09)	NS
NAA/mI	2.19 (0.35)	2.4 (0.29)	2.3 (0.29)	NS
Occipital lobe				
NAA	133.3 (23.1)	**133.7 (25.1)**	**146.9 (24.4)**	**p=0.03**
NAA/Cr	1.65 (0.08)	**1.49 (0.08)**	**1.6 (0.08)**	**p<0.0001**
Ch/Cr	0.6 (0.07)	0.55 (0.05)	0.57 (0.07)	NS
mI/Cr	0.65 (0.1)	0.59 (0.06)	0.6 (0.07)	NS
NAA/mI	2.52 (0.36)	2.63 (0.29)	2.61 (0.4)	NS

Table 2- Metabolite levels and ratios for the two areas explored. Statistical significance refers to the differences found between converters and non-converters.

FIGURE LEGENDS

Figure 1. A. Axial T2-weighted MRI.Voxel placement in the left occipital lobe. B. Sagittal T1-weighted MRI. Voxel placement in the posteromedial parietal cortex bilaterally.

Figure 2. Example of spectrum in the paretal lobe. NAA: N-acetyl-aspartate; Ch: choline compounds; Cr: creatine; mI: myo-Inositol.

Figure 3. ROC curves for the variables NAA/Cr in both areas explored. NAA_CRPC means posteromedial parietal cortex, and NAA_CRO means occipital lobe.

Figure 4. Dot plot depicting the NAA/Cr ratios in the posteromedial parietal cortex for converters and non-converters.

Figure 5- Dot plot depicting the NAA/Cr ratios in the left occipital lobe for converters and non-converters.

Version 2: BMJ Open-2010-000007

Title: **Magnetic Resonance Spectroscopy in the prediction of early conversion from amnestic Mild Cognitive Impairment to dementia.**

Authors: Pedro J Modrego[a], Nicolas Fayed,[b] Manuel Sarasa[c]

c) Department of Neurology. Hospital Universitario Miguel Servet. Zaragoza. Spain
d) Department of Radiology. Clinica Quiron. Zaragoza. Spain
c) Laboratory of Neurobiology. University of Zaragoza. Spain

Correspondence:

Dr PJ Modrego, MD, FEBN

Department of Neurology

Hospital Miguel Servet

50009 Zaragoza

Spain

e-mail: pmodrego@salud.aragon.es

ABSTRACT

Background. Mild Cognitive Impairment (MCI) of amnestic type is a common condition in the elderly and highly predictive of Alzheimer's disease (AD). To date there is no clear consensus regarding the best antecedent biomarker to predict early conversion to AD.

Objective. The aim of this study is to demonstrate that ^{1}H Magnetic Resonance Spectroscopy (MRS) of the brain in MCI patients may predict early conversion to dementia within the 2 year period after baseline assessment.

Methods. A cohort of patients fulfilling the criteria of amnestic MCI were enrolled consecutively. At baseline we carried out neuropsychological examination, Standard Blood tests, and APOE4 genotype. 1.5T ^{1}H-Magnetic Resonance Spectroscopy of the brain was carried out by exploring two areas: posteromedial bilateral parietal lobe and left medial occipital lobe.

The patients were followed-up to detect conversion to probable Alzheimer's disease according to the NINCDS-DRDA group criteria.

RESULTS. After 2 year follow-up, 27 (38%) patients converted to AD. The mean NAA/Cr ratio in the posteromedial bilateral parietal cortex was 1.38 in converters versus 1.49 in non-converters ($p<0.0001$). A NAA/Cr ratio equal to or lower than 1.43 in this area predicted conversion to probable AD at 74.1% sensitivity and 83.7% specificity (area under the curve: 0.84; 95% CI: 0.73-0.92). The cross-validated accuracy of classification was 82%, which reaches an 85% when APOE4 genotype and memory test are included in the analysis. In the left medial occipital lobe the predictive value was somewhat lower with 85.2% sensitivity and 61.4% specificity (area under the curve: 0.8; 95% CI: 0.69-0.89). Neither APOE4 genotype nor leuko-araiosis were predictive of conversion to dementia.

CONCLUSION. MRS is a valuable biomarker to predict early conversion to dementia in patients with amnestic MCI.

Key words: Mild Cognitive Impairment. Magnetic Resonance Spectroscopy.

SUMMARY SECTION

Article focus

Amnestic mild cognitive impairment (MCI) is a common condition at increased risk of conversion to Alzheimer's disease. There is no clear consensus regarding the ideal antecedent biomarker to predict early conversion.

There are a few longitudinal studies using brain Magnetic Resonance Spectroscopy (MRS) as predictor of conversion to dementia.

We hypothesize that MRS in MCI may identify the patients at risk of early conversion to dementia.

Key messages

Magnetic Resonance Spectroscopy is a reliable biomarker of AD and predicts early conversion to dementia in MCI.

Both posteromedial parietal and occipital regions were predictive of conversion but the parietal region was better in terms of accuracy of classification.

Neither APOE4 genotype nor leuko-araiosis were predictive of conversion.

Strengths and limitations.

This is a longitudinal study with is a non-invasive, reproducible, and widely available tool. However this technique is not free of artefacts limiting the accuracy of metabolite levels.

This study is based on early predictions (2 years from baseline). At longer term it is likely that all patients with objective memory impairment will convert to dementia.

Amnestic Mild cognitive impairment (MCI) is a common condition in the elderly mainly characterized by memory loss. Although there may be other subtle inefficiencies however the general cognitive function and daily living activities are preserved [1]. The meaning of this concept varies across the scientific community: a transitional state between normality and dementia [1], an early phase of Alzheimer's disease (AD) [2], or an unstable condition that may evolve to dementia or may even revert to normality [3]. Regardless of conceptualization most patients convert to AD over time but some of them remain non-demented. Meta-analysis from clinical trials in MCI have revealed that treatment with cholinesterase inhibitors does not delay the onset of AD [4, 5]. However in patients with mild to moderate AD cholinesterase inhibitors can delay cognitive decline and deterioration in global health for at least 6 months [6]. Given that it is not cost-effective to treat all MCI patients, we need a biomarker to predict conversion to dementia to start treatment as soon as possible in those at high risk.

Since brain pathology starts long before symptoms in AD many works have focused on antecedent biomarkers of AD to diagnose this disease in the early phase called MCI or even before. It is true that the atrophy of the medial temporal lobe structures, such as entorhinal cortex and hippocampus, has reached encouraging results [7-16], and CSF biomarkers (tau and Abeta42 proteins) as well [17-24] with large intra and inter-individual variations. So far no standardised procedures have been established.

Studies with PET in MCI and AD show the areas involved in early phases. The posterior cingulate gyrus (PCG) is an area involved in memory and many times studied in MCI and AD. The patients with AD had lower glucose metabolism than healthy controls in parietal, temporal, occipital, frontal and posterior cingulate cortices [25]. FDDNP-PET (radiotracer binding to plaques and tangles) [26] and FDG-PET [27] findings can discriminate normality from MCI and AD with the PCG being one of the most typically affected areas. In longitudinal studies PET has yielded the highest

accuracies to predict conversion to dementia but most studies included small cohorts of MCI patients [28-31].

Several cross-sectional studies with Magnetic Resonance Spectroscopy (MRS) found decreased N-acetyl-aspartate/ Creatine (NAA/Cr) ratios and increased Myo-inositol/Creatine (mI/Cr) ratios in the PCG of MCI and AD patients in comparison with controls [32-35], and in the occipital lobe of AD patients in comparison with controls [36, 37] and vascular dementia [38].

Longitudinal studies with MRS are scarce. In a cohort with 53 MCI patients the occipital NAA/Cr ratios but not those of the hippocampus and midparietal lobe were predictive of conversion to dementia with high accuracy [39]. In another cohort of 119 MCI patients compared the NAA/Cr ratios in the left occipital lobe with those obtained in the posteromedial parietal cortex (PMPC) yielding similar predictive values, with the PMPC values being a little more valuable than those observed in the occipital lobe. This study included amnestic and multiple domain MCI. At baseline it was observed a significant correlation between the ratios observed in these two locations [40]. Another small cohort (25 MCI patients) study showed that the NAA/Cr ratios in the left paratrigonal area were also predictive of conversion to dementia [41]. In a large cohort of 151 MCI patients (most being of amnestic type) followed-up for 3 years, MRS was individually predictive of conversion to dementia but the accuracy of prediction improved when MRS was used in combination with hippocampal volumetry and the presence of cortical infarctions [42]. In a small cohort of MCI (15) patients and controls (12) the ratios of NAA/Cr in the parietal lobe decreased longitudinally more in patients who converted to dementia than in non-converters [43].

On the basis of all of the above and, given the paucity of longitudinal MRS studies in MCI, the purpose of this work is to investigate whether MRS measuring cerebral baseline NAA/Cr ratios in amnestic MCI are predictive of early conversion to dementia. We hypothesize that the occipital and parietal values are similarly predictive of conversion to dementia.

PATIENTS AND METHODS

A cohort of patients fulfilling the criteria of amnestic MCI according to the Petersen et al [1] were recruited consecutively. The patients were referred by family physicians because of memory complains corroborated by an informant. Those included in our cohort were firstly screened for memory impairment with the Memory Impairment Screen (MIS) [44]. In the MIS, 4 written words are presented to the patient who must read aloud and memorize. After a spam of 5 minutes the patient is asked to recall these words; 2 points are given for every word recalled spontaneously, and

one point for every word recalled with cues. At baseline the patients underwent neuropsychological analysis encompassing Mini-Mental test (Spanish version with a maximum possible score of 35 points) [45], Blessed Dementia Rating Scale, clock drawing test, GDS (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 minute delayed recall were as follows: =/< 4 for patients aged up to 69, and =/<3 for the patient aged 70 and older. Those who scored 11 points or higher in the GDS were re-evaluated after antidepressant treatment so as to confirm that they had MCI.

The patients fulfilling the criteria mentioned above underwent also standard blood tests including B12 vitamin, serologic test of syphilis, and thyroid hormones. APOE genotype was also carried out. Brain magnetic resonance techniques were also carried out as follows. All patients underwent brain T1and T2-weighted MRI on a 1.5 T clinical scanner (Signa HD, GE). Single-voxel ^{1}H-MRS was carried out by means of an echo time (TE) of 35 milliseconds and a repetition time (TR) of 2000 milliseconds with spin echo technique that uses selective excitation with gradient spoiling for 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; 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 GE software (Signa HD, GE software release 12.x), version 3.0, 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 volume voxel was 2x2x2 cm in one of every area explored. These areas of exploration were the left medial occipital lobe and the posteromedial parietal area bilaterally encompassing the posterior cingulated gyrus and the inferior precuneus (see figure 1). Spectra were rejected and repeated in the following cases: linewidth >10 Hz, lineshape asymmetric after eddy current correction, and the presence of artifacts. Data fits with %SD>20 from the Cramer-Rao inequality were eliminated.

Both areas we examined showed excellent reproducibility in two previous studies of test-retest reliability done with the same clinical scanner in AD patients [46, 47]. For the NAA/Cr ratios the alpha 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. In spite of these good alpha values seen previously we also carried out a second immediate MRS in 22 patients without being removed from the scanner to check reproducibility. The intra-class correlation coefficients were 0.92 and 0.9 for parietal and occipital lobes respectively.

At baseline we also carried out MRS in 35 healthy elderly controls with the voxels located in the same areas for comparison purposes. The subjects were healthy volunteers that accepted participation to establish a normative group. The mean age was 70.3 years (SD: 7.8 y), and there were 23 women and 12 men.

The patients were followed-up and re-evaluated every 6 months or earlier to check if they converted to dementia of probable AD type according to the NINCDS-ADRDA group criteria [48]. Re-assessment was based on Mini-mental, MIS, BDRS, and clock drawing test.

Statistical analysis

The comparison of quantitative variables such as metabolite values in converters and non-converters was made with two-tailed t-tests. Survival analysis was based on Kaplan-Meier method and Cox proportional hazards model. According to the metabolite values found in MRS we divided the patients into two groups: those with NAA/Cr ratios below the average and those with values on average and higher. The proportions of patients free of conversion to dementia in each group were compared with the Log-Rank test. The risk of conversion to dementia was adjusted for potential confounders such as age, educational level, global cognitive function at baseline, memory, and APOE genotype with the Cox regression model.

The predictive values of the different variables (APOE4, memory tests, and brain metabolite values) were calculated with the analysis of ROC curves. Parameters such as sensitivity, specificity, positive and negative predictive values, and accuracy of classifications are reported. The results were cross-validated with discriminant analysis and leave-one-out technique. ROC curves were analyzed with Med-Calc software, and the other statistical techniques with the SPSS software, version 10.

We obtained informed consent from patients and relatives. This study was approved by our regional ethical committee.

RESULTS

Initially we recruited a cohort of 78 patients scoring 5 or lower in the MIS and that fulfilled the criteria of amnestic MCI. However MRS was not possible in 6 cases; 3 had claustrophobia, two bore pacemaker, and one refused participation. One patient had to be excluded because an incidental brain-stem tumor. Therefore 71 patients were finally included in the study. In table 1 are reported the main baseline demographic variables and the results of memory tests and scales. There were no differences with regard to female/male ratio between patients and controls, but the controls were somewhat younger than the patients (mean: 70.3 years versus 74; p=0.01). The mean age of the seven excluded patients was 76,4 years.

At baseline MRI (T1,T2, and FLAIR sequences) we detected the following abnormalities: diffuse cortical atrophy in 40 patients, isolated hippocampal atrophy in 9 patients, leuko-araiosis in 38 patients, and microinfarctions in 6 patients. Atrophy was evaluated in a visual way only.

After a mean follow-up of 22 months (range: 6-34 months), 27 (38%) patients out of 71 converted to probable AD according to the NINCDS-ADRDA criteria, and none of them reverted to normal. None of the converters showed symptoms or signs of parkinsonism, hallucinations, cognitive fluctuations, or focal symptoms. No differences were seen in the proportion of male/female ratio, but converters were older than non-converters [mean age: 76 (SD: 6.5) years for converters versus 73 (SD: 5.8) years for non-converters; p=0.01].

Spectroscopic results.

Controls versus MCI patients

In table 2 are reported the values of the different variables for converters and non-converters, and also for controls. When we compared the NAA/Cr ratios in the occipital lobe of the 71 MCI patients with 35 controls we saw significant differences. It was 1.56 (SD: 0.09) in patients in comparison with 1.65 (SD: 0.08) in controls (t= 5; p<0.0001). No significant differences were seen in the rest of metabolite ratios. In the posteromedial parietal cortex no global differences were observed in the mean NAA/Cr ratios which was 1,46 for controls and 1,45 for MCI patients. The differences in the NAA levels were significant: 134.06 (SD: 18.3) for controls versus 123.17 (SD: 17,3) for MCI patients (t=2.99; p=0.003). We did not see significant differences for the other metabolites and ratios.

Converters versus non-converters.

The mean NAA/Cr ratio in the posteromedial cortex was 1.30 (SD: 0.09) in converters versus 1.49 (SD: 0.08) in non-converters (t=9.96; p<0.0001). In the occipital lobe it was 1.48 (SD:

0.08) in converters versus 1.6 in non-converters (t=4, 89; p=0.0001). The absolute occipital NAA level was 133.7 (SD: 25.1) in converters versus 146.9 (SD: 24.4) in non converters (p=0.03). Figures 2a and 2b present an example in a non-converter and in a converter respectively. The differences were not significant for the other metabolite values (see table 2). It is worth mentioning that the baseline NAA/Cr values in the posteromedial and occipital cortices correlated significantly (r=0.56; p<0.0001) in the whole sample of 71 patients.

In the survival analysis we saw significant differences in the proportion of dementia-free patients at follow-up (see figures 3 and 4). The patients with NAA/Cr ratios below average were more likely to convert to dementia than those with values above average in both posteromedial parietal (log-rank test: 17.83, p<0.0001) and occipital lobe (log-rank test: 11.7; p=0.0007). The differences were adjusted for potential confounders (age, educational level, global cognitive function, memory scale, and APOE genotype) with the Cox regression model. Only global cognition (MEC) and memory scale (RAVLT) at baseline were also predictive of conversion to dementia. The adjusted hazard ratio (HR) for the NAA/Cr ratios below average in the posteromedial parietal lobe was 7.03 (95% CI: 2.6-18.9). In the occipital lobe the HR was 5.06 (96% CI: 1.73-14.8). The results were also predictive when NAA/Cr ratios were factored out as continuous variables.

A NAA/Cr ratio equal to or lower than 1.43 in the posteromedial bilateral parietal cortex predicted conversion to probable AD at 74.1% sensitivity and 83.7% specificity, with a positive predictive value of 74.1% and a negative predictive value of 83.7%. The area under the curve was 0.84 (95% CI: 0.73-0.92). The cross-validated accuracy of classification was 82%, which reaches an 85% when APOE genotype and memory test are included in the analysis. In the left medial occipital lobe the predictive value was somewhat lower with 85.2% sensitivity, 61.4% specificity, a positive predictive value of 57.5%, and a negative predictive value of 87.1%. The area under the curve was 0.8 (95% CI: 0.69-0.89). The ROC curves are presented in figure 5.

The APOE4 genotype alone yielded low predictive values in terms of sensitivity as 18 patients converted to dementia despite not having APOE4 alleles and only 8 of converters had one or two alleles (33% sensitivity and 72% specificity). The RAVLT yielded low sensitivity but high specificity (55.6% and 84% respectively). The presence/absence of white matter hyperintensities (leuko-araiosis) was not predictive of conversion to dementia.

.

DISCUSSION

The diagnosis of AD in early phases is still challenging. Several tools, clinical and radiological, have been used so far but there is not a consensus on what the best is. In addition, determined techniques are not available in every medical center, so each department should take advantage of the available ones.

MRS represents a valuable technique in AD on the basis of previous cross-sectional and longitudinal published works. Additionally it was found that MRS correlates well with histopathology. The NAA levels were lower in the brain of AD than in controls and that this decrease correlated with the number of neuritic plaques and neurofibrillary tangles in tissue sections [49]. The value of proton MRS as a biomarker has also been assessed ante-mortem in a series of 54 patients ranging from low to high likelihood of having AD and who underwent autopsy. Decreases in NAA/Cr and increases in mI/Cr ratios correlated with higher Braak neuropathological stages in the posterior bilateral cingulate gyrus [50]. Furthermore, the value of MRS as biomarker has been confirmed with the fact that changes in metabolite ratios are detected years before the clinical onset of AD in subjects carrying mutations in presenilins [51] or protein tau [52] genes.

The predictive NAA/Cr ratios of the posteromedial parietal lobe agree with the early involvement of the PCG in AD but it may result surprising in the occipital lobe as histopathology in this region appears in more advances stages of the disease. However two cross-sectional studies with MRS showed lower NAA/Cr ratios in AD patients than in controls in the occipital cortex [36, 37], and one study in MCI showed lower NAA/Cr ratios in converters than in converters [39]. Two more studies point to an earlier involvement than thought of the occipital cortex in AD. A PET study in 13 patients with mild to moderate AD disclosed that glucose metabolism correlated to cognitive performance in the parietal lobes, but for the activation condition, the authors also found correlations within the primary and association visual areas [53]. A neuropathological study revealed dense AD pathology in area 19 in some subjects with preclinical AD and in all patients with MCI, and noted that it was present even in the absence of hippocampal and entorhinal pathology [54].

Apart from the intrinsic predictive value of a biomarker it should be weighted in comparison with other biomarkers available. On the one hand, volumetry of the medial temporal lobe structures is widely used but not free of limitations (artifacts, lack of standardization, complexity and long duration of the process). On the other hand, the CSF biomarkers need an invasive procedure and admission to hospital. Furthermore these biomarkers do not differ greatly from MRS in terms of accuracy of prediction. At a fixed

80% specificity the hippocampus plus entorhinal cortex volume predicted conversion to AD at 66.7% sensitivity in a cohort of 139 MCI patients followed-up for 5 years [16]. According to a large prospective prospective multicenter cohort results with 750 MCI subjects followed-up for a minimum of 2 years, the combination of Aβ42/P-tau ratio and T-tau in the CSF predicted conversion to AD with a sensitivity of 83%, a specificity of 72%, a positive predictive value of 62% and a negative predictive value of 88%, but the analytical techniques are pending standardization [24]. PET appears to be a robust predictor of conversion to dementia but it is expensive and of limited availability. The excellent values found in the previous studies with PET [28-31] have not been confirmed in the cohort of the AD Neuroimaging Initiative group (ADNI) where the positive predictive valued was 41% and the negative one was 79% [55].

PET with Pittsburg radiotracer (amyloid plaques binding) also seems promising in terms of specificity. In a study including 31 MCI patients as 14 (82% of PIB positive patients) converted to dementia over 3 years. However, only 17 MCI patients (55%) were PIB positive at baseline [56].

In this context of expensive and sophisticated techniques it should be borne in mind that neuropsychological tests can disclose good predictions of conversion to dementia in experienced hands [57].

Of course MRS has also some shortcomings. Firstly, with the large voxels analysed, it is sensitive to artifacts in the magnetic field and partial volume effect in areas next to osseous structures and cerebral ventricles [58]. For this reason it is likely that in our previous cohort we did not find predictive values in the hippocampus although this area is theoretically involved very early in AD. It is expected that modern 3T scanners with smaller voxel analysed will overcome these limitations [59, 60]. Secondly, quantification of absolute metabolite values is complex so the metabolite ratios to creatine are much more reliable than the absolute levels as they can minimize systematic errors [61]. We can see an example in table 2 the controls had lower NAA levels in the occipital lobe than non-converters whereas the NAA/Cr ratios were higher in controls as it is expected to occur. Thirdly, we were not able to make corrections for atrophy and CSF.

In conclusion MRS is a useful technique as biomarker in early Alzheimer's disease as it predicts early conversion to dementia. Although inferior to FDG-PET, it could yield similar performance to structural neuroimaging and CSF biomarkers. We think MRS may play a role where no better instruments are available.

ACKNOWLEDGEMENTS

This work was supported by the Spanish Ministry of Education and Science (SAF 2006-13332). None of the authors has conflicts of interest to declare.

AUTHORS CONTRIBUTION

Dr PJ Modrego: design, clinical data acquisition. Statistical analysis. Drafting of the manuscript.

Dr N Fayed: Magnetic Resonance Imaging acquisition. Design. Critical review.

Dr M Sarasa: acquisition of funding. APOE4 genotype determination. Critical review.

GUARANTOR OF THE STUDY: Dr PJ Modrego

REFERENCES

1. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild Cognitive Impairment. Clinical characterization and outcome. Arch Neurol 1999; 56: 303-308.

2. Morris JC, Storandt M, Miller JP, et al. Mild Cognitive Impairment represents early-stage Alzheimer's disease. Arch Neurol 2001; 58: 397-405.

3. Larrieu S, Letenneur L, Orgogozo JM, et al. Incidence and outcome of mild cognitive impairment in a population based cohort. Neurology 2002; 59: 1594-1599.

4. Birks J, Flicker L. Donepezil for mild cognitive impairment. Cochrane Database Syst Rev 2006; 19; 3: CD006104

5. Raschetti R, Albanese E, Vanacore N, Maggini M. Cholinesterase inhibitors in mild cognitive impairment: a systematic review of randomised trials. PLoS Med 2007: 4 (11): e338

6. Wolfson C, Oremus M, Shukla V, et al. Donepezil and rivastigmine in the treatment of Alzheimer's disease : a best evidence synthesis of the published data on their efficacy and cost-effectiveness. Clin Ther 2002; 24: 862.886

7. De León MJ, Golomb J, George AE, et al. The radiologic prediction of Alzheimer's disease: The atrophic hippocampal formation. Am J Neuroradiol 1993; 14; 897-906.

8. Jack CR, Petersen RC, Xu YC, et al. Prediction of AD with MRI based hippocampal volume in mild cognitive impairment. Neurology 1999; 52: 1397-1403

9. Killiany RJ, Gómez-Isla T, Moss M et al. Use of structural magnetic resonance imaging to predict who will get Alzheimer's disease. Ann Neurol 2000; 47: 430-439.

10-Dickerson BC, Goncharova I, Sullivan MP et al. MRI-derived entorhinal and hippocampal atrophy in incipient and very mild AD. Neurobiol Aging 2001; 22: 747-754.

11-Visser PJ, Verhey FR, Hofman PA, Scheltens P, Jolles JP. Medial temporal lobe atrophy predicts Alzheimer's disease in mild cognitive impairment. J Neurol Neurosurg Psychiatry 2002; 72: 491-497.

12. Korf ES, Wahlund LO, Visser PJ, Scheltens P. Medial temporal lobe on MRI predicts dementia in patients with mild cognitive impairment. Neurology 2004; 63: 94-100.

13. De Toledo-Morrell L, Stoub TR, Bulgakova M, et al. MRI-derived entorhinal volume is a good predictor of conversion from MCI to AD. Neurobiol Aging 2004; 25: 1197-1203.

14. Jack CR, Shiung MM, Weigand SD, et al. Brain atrophy rates predict subsequent clinical conversion in normal elderly with amnestic MCI. Neurology 2005; 65: 1227-1231.

15. Stoub TR, Bulgakova M, Leurgans S, et al. MRI predictors of risk of incident Alzheimer's disease: a longitudinal study. Neurology. 2005; 64: 1520-1524.

16. Devanand DP, Pradhaban G, Liu X, et al. Hippocampal and entorhinal atrophy in mild cognitive impairment : prediction of Alzheimer disease. Neurology 2007; 68: 828-836.

17. Riemenschneider M, Lautenschlager N,Wagenpfeil S, Diehl J, Drzezga A, Kurz A. Cerebrospinal fluid tau and beta-amyloid 42 proteins identify Alzheimer's disease in subjects with mild cognitive impairment. Arch Neurol. 2002; 59: 1729-1734.

18. Zatterberg H, Wahlung LO, Blennow K. Cerebrospinal fluid markers for prediction of Alzheimer's disease. Neurosci Lett 2003; 352-67-69.

19. Andreasen N, Vanmechelen E, Vanderstichele H, Davidsson P, Blennow K. Cerebrospinal fluid levels of total-tau, phospho-tau and A Beta42 predicts development of Alzheimer's disease in patients with mild cognitive impairment.Acta Neurol Scand 2003; 179: 47-51.

20. Hampel H,Teipel SJ, Fuchrberger T, et al. Value of CSF-Beta-amyloid1-42 and tau as predictors of Alzheimer's disease in mild cognitive impairment. Mol Psychiatry 2004; 9:705-710.

21. Ivanoiu A, Sindic CJ. Cerebrospinal fluid Tau protein and Amyloid-B42 in mild cognitive impairment: prediction of progression to Alzheimer's disease and correlation with the neurological examination. Neurocase 2005; 11: 32-39.

22. de Leon MJ, Segal S, Tarshish CY, et al. Longitudinal cerebrospinal fluid tau load increases in mild cognitive impairment. Neurosci Lett 2002; 333-183-186.

23. Vanderstichele H, De Meyer G, Andreasen N, et al. Amino-truncated {beta}-Amyloid42 peptides in cerebrospinal fluid and prediction of progression of mild cognitive impairment. Clin Chem 2005; 51: 1650-1660.

24. Mattsson N, Zetterberg H, Hansson O, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. JAMA 2009; 302: 385-393.

25. Alexander GE, Chen K, Pietrini P, Rapoport SI, Reiman EM. Longitudinal PET evaluation of cerebral metabolic decline in dementia : A potential outcome measure in Alzheimer's disease treatment studies. Am J Psychiatry 2002; 159: 738-745.

26. Small GW, Kepe V, Ercoli LM, et al. PET of brain amyloid and tau in mild cognitive impairment. New Engl J Med 2006; 355: 2652-2663.

27. Hunt A, Schonknecht P, Henze M, Seidl U, Haberkorn U, Schröder J. Reduced cerebral glucose metabolism in patients at risk for Alzheimer's disease. Psychiatry Res 2007; 155: 147-154.

28. de Leon MJ, Convit A, Wolf OT, et al. Prediction of cognitive decline in normal elderly subjects with 2-[(18)F]fluoro-2-deoxy-D-glucose/positron-emission tomography (FDG/PET). Proc Natl Acad Sci USA 2001; 98: 10966-10971.

29. Mosconi L, Perani D, Sorbi S, et al. MCI conversion to dementia and the APOE genotype: a prediction study with FDG-PET. Neurology 2004; 63: 2332-2340

30. Drzezga A, Grimmer T, Riemenschneider M, et al. Prediction of individual clinical outcome in MCI by means of genetic assessment and (18)F-FDG PET. J Nucl Med 2005; 46: 1625-1632.

31. Anchisi D, Borroni B, Franceschi M, et al. Heterogeneity of brain glucose metabolism in mild cognitive impairment and clinical progression to Alzheimer's disease. Arch Neurol 2005; 62: 1728-1733.

32. Kantarci K, Petersen RC, Boeve BF, et al. ^{1}H MR spectroscopy in common dementias. Neurology 2004; 63: 1393-1398.

33. Kantarci K, Jack CR, Xu YC, et al. Regional metabolic patterns in mild cognitive impairment and Alzheimer's disease. Neurology 2000; 55: 210-217.

34. Hancu I, Zimmerman EA, Sailasuta N, Hurd RE. H MR spectroscopy using TE averaged PRESS: A more sensitive technique to detect neurodegeneration associated with Alzheimer's disease. Magn Reson Med 2005; 53: 777-782.

35. Kantarci K, Xu YC, Shiung MM, et al. Comparative diagnostic utility of different MR modalities in Mild Cognitive Impairment and Alzheimer's disease. Dement Geriatr Cogn Disord 2002; 14: 198-207

36. Miller BL, Moats RA, Shonk T, Earnst E, Wooley S, Ross BD. Alzheimer's disease: depiction of increased cerebral myoinositol with proton MR spectroscopy. Radiology 1993; 187:433-437.

37. Shonk TK, Moats RA, Gifford P, Michaelis T, Mandigo JC, Izumi J, Ross BD. Probable Alzheimer Disease: diagnosis with Proton MR Spectroscopy. Radiology 1995; 195: 65-72.

38. Rai GS, McConnel JR, Waldman A, Grant D, Chaudhry M. Brain proton spectroscopy in dementia: an aid to clinical diagnosis. Lancet 1999; 353: 1063-1064.

39. Modrego PJ, Fayed N, Pina MA. Conversión from Mild Cognitive Impairment to probable Alzheimer's disease predicted by brain Magnetic Resonance Spectroscopy. Am J Psychiatry 2005; 162: 667-675.

40. Fayed N, Davila J, Oliveros A, castillo J, Medrano JJ. Utility of different MR modalities in mild cognitive impairment and its use as a predictor of conversión to probable dementia. Acad Radiol 2008; 15: 1089-1098.

41. Metastasio A, Rinaldi P, Tarducci R, et al. Conversión of MCI to dementia: Role of proton magnetic resonance spectroscopy. Neurobiol Aging 2006; 27: 926-932.

42. 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

43. Pilatus U, Lais C, Rochmont Adu M, et al. Conversion to dementia in mild cognitive impairment is associated with decline of N-acetylaspartate and creatine as revealed by magnetic resonance spectroscopy. Psychiatry Res 2009; 173: 1-7.

44. Buschke H, Kuslansky G, Katz M, Stewart WF, Slwinski MJ, Eckholdt HM, Lipton RB. Screeing for dementia with the memory impairment screen. Neurology 1999; 52: 231-238.

45. Lobo A, Ezquerra J, Gomez Burgada F, Sala JM, Seva Diaz A. El Mini-Examen cognoscitivo: un test sencillo y practico para detectar alteraciones intelectuales en pacientes médicos. Actas Luso Esp Neurol Psiquiatr Cienc Afines 1979; 7:189-202.

46. 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: 472-477.

47. Modrego PJ, Fayed N, Errea JM, Rios C, Pina MA, Sarasa M. Memantine versus donepezil in Alzheimer's disease. A randomized trial with Magnetic Resonance Spectroscopy. Eur J Neurol 2010; 17: 405-412.

48. McKhan G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group. Neurology 1984; 34: 939-944.

49. Klunk WE, Panchalingam K, Moossy J, McClure RJ, Pettegrew JW. N-acetyl-L-aspartate and other amino acid metabolites in Alzheime's disease brain: a preliminary proton nuclear magnetic resonance study. Neurology 1992: 42: 1578-1585.

50. Kantarci K, Knopman DS, Dickson DW, et al. Alzheimer disease: Postmortem neuropathologic correlates of antemortem 1-H MR Spectroscopy metabolite measurements. Radiology 2008; 248: 210-220.

51. Godbolt AK, Waldman AD, MacManus DG, Schott JM, Frost C, Cipolotti L, et al. MRS shows abnormalities before symptoms in familial Alzheimer disease. Neurology 2006; 66:718-722.

52. Kantarci K, Boeve BF, Wszolek Z, et al. MRS in presymptomatic MAPT mutation carriers. Neurology 2010: 75: 771-778

53. Bokde AL, Teipel SJ, Drzezga A, et al. Association between cognitive performance and cortical glucose metabolism in patients with mild Alzheimer's disease. Dement Geriatr Cogn Disord 2005; 20: 352-357.

54. McKee AC, Cabral HJ, Kowall NW, et al. Visual association pathology in preclinical Alzheimer disease. J Neuropathol Exp Neurol 2006; 65: 621-630.

55. Landau SM, Harvey D,Madison CM, et al. Comparing predictors of conversion and decline in mild cognitive impairment. Neurology 2010; 75: 230-238

56. Okello A, Koivunen J, Edison P, et al. Conversión of amyloid positiveand negative MCI to AD over 3 years: an 11C-PIB PET study. Neurology 2009; 73:754-760

57. Modrego PJ. Predictors of conversion to dementia of probable Alzheimer type in patients with mild cognitive impairment. Curr Alzh Res 2006; 3: 161-170.

58. Fayed N, Olmos S, Morales H, Modrego PJ. Physical basis of Magnetic Resonance Spectroscopy and its application to Central Nervous System diseases. Am J Appl Sci 2006; 3: 1836-1845.

59. Wang Z, Zhao C, Yu L, Zhou W, Li K. Reginal metabolic changes in the hippocampus and posterior cingulated area detected with 3-Tesla Magnetic resonance spectroscopy in pateients with mild cognitive impairment and Alzheimer's disease. Acta Radiol 2009; 50: 312-319.

60. Caserta MT, Ragin A, Hermida AP, John Ahrens R, Wise L. Single voxel MR spectroscopy at 3T in a memory disorders clinic: early right hippocampal NAA/Cr loss in mildly impaired subjects. Psychiatry Res 2008; 164: 154-159.

61. Danielsen ER, Ross B. Basic physics of MRS. In: Danielsen ER, Ross B, eds. Magnetic Resonance Spectroscopy diagnosis of neurological diseases. New York, Marcel Dekker 1999: 5-22

Variables (n=71)

AGE: 74.6 y (SD: 6.4; range: 58-88)

SEX: 43 were female

MEC: 28.4 (SD: 3.1; range: 21-34)

BDRS: 2.8 (SD: 0.9; range: 1-4)

MIS: 2.4 (SD: 1.7; range: 0-5)

RAVLT: 3.1 (SD: 2.5; range: 0-6) in delayed recall.

Elementary education: 65 patients

High school education: 6 patients

Higher education: 4 patients

Hypertension: 24 (33.8%)

APOE4 genotype: 21 had 1 or two alleles

Mean follow-up: 22 months (range: 6-34 months).

Mean number of visits: 4.4 (range 3-7).

Table 1. Demographic variables and scales scores in the cohort of 71 patients with amnestic MCI.

VARIABLE	Controls n=35	Converters N=27	Non-converters N=44
Posteromedial parietal cortex			
NAA	134 (18.3)	120 (19.89	125 815.59 NS
NAA/Cr	1.46 (0.08)	**1.38 (0.09)**	**1.49 (0.08) p<0.0001**
Ch/Cr	0.61 (0.07)	0.62 (0.05)	0.59 (0.1) NS
mI/Cr	0.66 (0.08)	0.63 (0.08)	0.6 (0.09) NS
NAA/mI	2.19 (0.35)	2.4 (0.29)	2.3 (0.29) NS
Occipital lobe			
NAA	133.3 (23.1)	**133.7 (25.1)**	**146.9 (24.4) p=0.03**
NAA/Cr	1.65 (0.08)	**1.49 (0.08)**	**1.6 (0.08) p<0.0001**
Ch/Cr	0.6 (0.07)	0.55 (0.05)	0.57 (0.07) NS
mI/Cr	0.65 (0.1)	0.59 (0.06)	0.6 (0.07) NS
NAA/mI	2.52 (0.36)	2.63 (0.29)	2.61 (0.4) NS

Table 2- Metabolite levels and ratios for the two areas explored. Statistical significance refers to the differences found between converters and non-converters.

FIGURE LEGENDS

Figure 1. A. Axial T2-weighted MRI.Voxel placement in the left occipital lobe. B. Sagittal T1-weighted MRI. Voxel placement in the posteromedial parietal cortex bilaterally.

Figure 2 a). Example of spectrum in the parietal lobe in a non-converter . NAA: N-acetyl-aspartate; Ch: choline compounds; Cr: creatine; mI: myo-Inositol.

b). Example of spectrum in a converter. The NAA peak is lower than in the previous example in relation to creatina.

Figure 3. Comparison of survival curves for the variable NAA/Cr in the posteromedial parietal cortex. The curves represent the proportion of patients free of dementia across the time in the patients with the NAA/Cr ratio equal to or higher than the mean (upper curve) and in those with ratios below the mean (lower curve).

Figure 4. Comparison of survival curves for the variable NAA/Cr in the left occipital lobe. Proportion of patients free of dementia across the time in the patients with the NAA/Cr ratio equal to or higher than the mean (upper curve) and in those with ratios below the mean (lower curve).

Figure 5. ROC curves for the variables NAA/Cr ratio in the posteromedial parietal cortex (continuous line), and in the left occipital lobe (discontinuous line). All predictive values are given in the text.