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COMPARATIVE DIAGNOSTIC UTILITY OF DIFFERENT MR MODALITIES IN MILD COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE

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Abstract

This study compares diagnostic accuracy of magnetic resonance (MR)-based hippocampal volumetry, single voxel (SV) ¹H MR Spectroscopy (MRS) and MR diffusion weighted imaging (DWI) measurements in discriminating patients with amnesic mild cognitive impairment (MCI), Alzheimer's disease (AD) and normally aging elderly. Sixty-one normally aging elderly, 24 MCI, and 22 AD patients underwent MR-based hippocampal volumetry, ¹H MRS, and DWI. ¹H MRS voxels were placed over both of the posterior cingulate gyri and N-acetyl aspartate (NAA) / creatine (Cr), myoinositol (MI) /Cr and NAA /MI ratios were obtained. Apparent diffusion coefficient (ADC) maps were derived from DWI and hippocampal borders were traced to measure hippocampal ADC. At 80% specificity, the most sensitive single measurement to discriminate MCI (79 %) and AD (86 %) from controls was hippocampal volumes. The most sensitive single measurement to discriminate AD from MCI was posterior cingulate gyrus NAA /Cr (67 %). At high specificity (>85 –90%) combinations of MR measures had superior diagnostic sensitivity compared to any single MR measurement for the AD vs. control and control vs. MCI comparisons. The MR measures that best discriminate more from less affected individuals along the cognitive continuum from normal to AD vary with disease severity. Selection of imaging measures used for clinical assessment or monitoring efficiency of therapeutic intervention should be tailored to the clinical stage of the disease.

Keywords

Alzheimer's disease; mild cognitive impairment; ¹H MRS; diffusion weighted imaging; hippocampal volumetry; MRI

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Introduction

Although no disease modifying treatment is currently available, recent advances in understanding the molecular biology of Alzheimer's disease (AD), particularly β -amyloid metabolism offer the promise of useful therapeutic intervention in the foreseeable future (1, 2). For this reason, improved methods for early diagnosis and clinical assessment of AD have become more imperative. The diagnosis of AD on clinical grounds is quite accurate in patients who manifest clear-cut, fairly advanced symptoms. However, early diagnosis (i.e. prediction of future development of AD) in mildly affected individuals is far more problematic (3, 4). The clinically definable syndrome of mild cognitive impairment (MCI, or more specifically amnesic MCI) was established in order to identify on clinical grounds, individuals who are at increased risk of progressing to AD (3, 4). Clinically, MCI resides between normal aging and AD along the cognitive continuum. People with MCI do not meet diagnostic criteria for AD; however, the majority of them convert to AD within six years (3). MCI patients, certainly those that ultimately progress to AD, are felt to have AD pathology in its early stages, clinically manifesting itself as isolated memory impairment (5–8). Demonstrating that an imaging study can accurately differentiate MCI patients from normal elderly can be reasonably regarded as validation of its utility for early detection of AD pathology. Similarly, demonstrating that an imaging study can discriminate MCI from AD indicates that the test may be useful in assessing the risk-rate for progression from MCI to AD (9).

Magnetic resonance (MR) techniques; MR-based hippocampal volumetry, MR diffusion weighted imaging (DWI), and ^1H MR spectroscopy (MRS) identify structural and biochemical alterations in the brains of MCI and AD patients (10–12). Based on the temporal course and the regional distribution of the neurofibrillary pathology in AD, that involves the limbic cortex earlier and more profoundly than the isocortex (13), quantitative MR measurements have been targeted to the limbic regions (14). MR-based volumetry has identified that the hippocampi of patients with MCI and AD are smaller than the hippocampi of normal elderly (10). DWI has revealed that the diffusivity of water is higher in the hippocampi of patients with MCI and AD than normal elderly people, suggesting an expansion of extracellular space due to neuron loss in the hippocampi of MCI and AD patients (12). ^1H MR Spectroscopy (MRS) demonstrates higher myoinositol (MI) / creatine (Cr) ratios and lower N-acetyl aspartate (NAA) /Cr in AD patients relative to normal elderly subjects (11, 15–19).

Each of these quantitative MR modalities contains useful information for early diagnosis of AD. However, except for one study, which investigated MRS measurements and hippocampal volumetry (19), nearly all published work has focused on the diagnostic ability of a single imaging technique, rather than on a comparison among several techniques. It is therefore useful to define the relative utility of these MR techniques for distinguishing both MCI and early AD patients from cognitively normal elderly subjects. The purpose of this study was to compare the diagnostic accuracy of MR-based hippocampal volumetry, single voxel (SV) ^1H MRS, and DWI measurements alone and combined, in discriminating among the three clinically definable groups that reside along the cognitive continuum: normally aging elderly, MCI and AD patients.

Methods

Recruitment and Characterization

Twenty-two probable AD, 24 MCI and 61 elderly control subjects were consecutively recruited from the Alzheimer's Disease Research Center (ADRC) /Alzheimer's Disease Patient Registry (ADPR) at the Mayo Clinic. These are IRB approved prospective longitudinal databases of aging and dementia (20). Informed consent for participation was obtained from every subject and/or an appropriate surrogate. A behavioral neurologist and a neuropsychologist evaluated

individuals participating in ADRC/ADPR. Neurological examination and neuropsychological testing that included Mini-Mental State Examination (MMSE) (21), Dementia Rating Scale (DRS)²², Wechsler Adult Intelligence Scale –Revised (WAIS-R) full scale IQ, Wechsler Memory Scale -Revised (WMS -R) logical memory II and visual reproduction II (23) were done. AD and MCI subjects underwent laboratory tests including a chest radiograph, ECG, chemistry profile, CBC count, thyroid function tests, vitamin B-12 level, folic acid level, syphilis serology. All of the subjects underwent structural brain MRI. At the completion of the evaluation, a consensus committee meeting was held involving the behavioral neurologists, neuropsychologists, nurses and the geriatrician who evaluated the subjects. Subjects with structural abnormalities that could produce dementia, cortical infarction, tumor, subdural hematoma and who had concurrent illnesses or treatments interfering with cognitive function other than AD were excluded. Subjects were not excluded for the presence of leukoaraiosis.

The subjects who were classified as probable AD fulfilled the Diagnostic and Statistical Manual for Mental Disorders 3rd edition – revised (DSM-III-R) (24) criteria for dementia, and the National Institute of Neurological and Communicative Disorders and Stroke / Alzheimer's Disease and Related Disorder's Association (NINCDS / ADRDA) (25) criteria for AD. The severity of dementia was rated with Clinical Dementia Rating (CDR) score (26). Fifteen of the probable AD patients were taking donepezil hydrochloride (HCl) (5–10 mg /day) for an average duration of 16 ± 6.66 months.

The operational definition of MCI was clinical. In general, MCI patients were defined by the following characteristics: 1) subjective memory complaint, 2) normal general cognitive function determined by tests of general intellectual function, 3) normal activities of daily living, 4) objective memory impairment, and 5) not demented. These patients had a CDR of 0.5.

Controls were defined as individuals who; 1) were independently functioning community dwellers 2) did not have active neurological or psychiatric conditions, 3) had no cognitive complaints, 4) had a normal neurological exam, 5) were not taking any psychoactive medications in doses that would impact cognition.

With ¹H MRS, it has previously been shown that MI /Cr ratios are higher in the brains of patients with diabetes mellitus (27). Therefore, nine otherwise eligible diabetic subjects (six controls, one MCI, and two AD) were excluded from this study.

All of the subjects underwent MRI, ¹H MRS, and DWI within one week of the clinical evaluation on which the clinical group classification was based. ¹H MRS and DWI findings from 53 of the controls, 21 of the MCI and 20 of the AD patients were previously reported in two separate manuscripts, each addressing only a single MR measure (11,¹²). Hippocampal volumes have not been previously reported.

MRI and Hippocampal Volumetry

The MRI, DWI and single voxel (SV) ¹H MRS studies were performed on a 1.5 T (Signa; General Electric Medical Systems, Milwaukee, WI) MR scanner. A standardized imaging protocol was used for hippocampal volume measurements (28). A T1 weighted sagittal sequence with five mm. contiguous sections was used for intracranial volume measurements. A T1 weighted three dimensional volumetric spoiled gradient recalled echo (3D-SPGR) sequence with 124 contiguous partitions, 1.6 mm slice thickness, 22 cm × 16.5 cm field of view, 192 views, and 45° flip angle was used for the volume measurements of the hippocampi. The scanning time was about 9 min.

The same investigator who was blinded to all clinical information (MS) performed image-processing steps, which took about three and a half hours for each subject's data. Ongoing

validation studies show that the intrarater, test-retest coefficient of variation of hippocampal volume measurements is 0.97 % with this method for this investigator (MS). The 3D-image data set of each patient was reformatted so that the image sections were oriented perpendicularly to the principal axis of the left hippocampal formation. The image data were then interpolated in plane to the equivalent of a 512×512 matrix and magnified two times. The hippocampal borders were manually traced on each slice sequentially from posterior to anterior using previously defined boundaries (28). The number of pixels in each slice was counted automatically by using a summed region of interest (ROI) function and then multiplied by voxel volume to give a numeric value in cubic millimeters.

SV -¹H MRS

¹H MRS studies were performed with the LX system automated single voxel MRS package: Proton Brain Examination /Single Voxel (PROBE /SV) (General Electric Medical Systems, Milwaukee, WI) (29). T₁ weighted images in sagittal and coronal planes were obtained for localizing the ¹H MRS voxel. Point resolved spectroscopy (PRESS) pulse sequence with TR = 2000 ms, TE=30 ms, 2048 data points and 128 excitations were used for the examinations. The prescan algorithm of PROBE automatically adjusted the transmitter and receiver gains and center frequency. The local magnetic field homogeneity was optimized with the three-plane auto-shim procedure, and the flip angle of the third water suppression pulse was adjusted for chemical-shift-water suppression (CHESS) prior to PRESS acquisition. The total scanning time for the T₁ weighted sagittal and coronal localizer images and the PROBE acquisition was about 10 min. We analyzed the metabolite intensity ratios, which were automatically calculated at the end of each PROBE /SV acquisition.

We have previously studied the regional metabolic patterns in the brains of normal elderly, MCI and probable AD subjects with ¹H MRS (11). Based on those previous findings, only the posterior cingulate gyrus NAA /Cr, MI /Cr and NAA /MI ratios were evaluated in the current study as these offered the best measure for discriminating the three clinical groups (normal elderly, MCI, and AD).

An eight cm³ (2×2×2cm) posterior cingulate VOI was prescribed on a mid-sagittal T1 weighted image. It was placed below the cingulate sulci and above the parietooccipital sulci, which covered both of the posterior parts of posterior cingulate gyri and inferior precuneus (figure 1). The VOI in each subject were placed in a uniform manner by the same investigator (KK).

DWI

Single shot echo planar - fluid attenuated inversion recovery (EPI-FLAIR) DWI was performed in a coronal plane with TR=10 s, TE=93ms, TI= 2200 ms, slice thickness = 5 mm, slice spacing = 2.5 mm, and field of view (FOV)= 40×20 cm. to cover whole head. A FLAIR image with b=0 s/mm², and DWI with b=1000 s/mm² in three orthogonal directions were acquired from each slice. The scanning time was about 5 min. With the image analysis software FuncTool (General Electric Medical Systems, Milwaukee, WI), average apparent diffusion coefficient (ADC) maps were computed pixel by pixel based on the Stejskal and Tanner equation (30).

In a previous study, we measured the ADC from eight different regions (right and left frontal, parietal, occipital, temporal stem, anterior and posterior cingulate white matter, thalami and hippocampi) in the brains of normal elderly controls, MCI and probable AD patients (12). Among the eight different regions, evaluated in the prior study (12, only the ADC of the hippocampi were used in the current study as this was the most promising measure for distinguishing normal controls, MCI, and probable AD patients.

The hippocampal ROI were manually traced in order to exclude the perihippocampal cerebrospinal fluid (CSF) spaces. Tracing was done over the EPI-FLAIR images ($b = 0 \text{ s/mm}^2$) that concurrently appeared on the ADC maps. Because the spatial resolution of the EPI-FLAIR images was low, coronal T1 weighted images that had identical slice thickness and location were used as an anatomic reference for the tracing of the ROI. The same investigator who was blinded to the clinical diagnoses of the subjects (KK) traced borders of the hippocampi, which took about 20 min for each subject's data.

Statistical Analyses

The differences in the mean ages, education (in years) and the MMSE scores in three clinical groups were tested by t-tests and the gender differences were tested by chi-square tests. Hippocampal volumes of every subject were normalized for intersubject variation in head size by dividing structure volume (mm^3) by the total intracranial volume (cm^3). Volumes were converted to normal deviates, referred to as W scores, using age and gender specific normal percentile based on a previous study (10). The right and left hippocampal ADC were summed for the analyses. The effects of age on NAA /Cr, NAA /MI, MI /Cr ratios and hippocampal ADC and normalized volumes were tested using regression analysis in control subjects only, as the data in controls should be free of the confounding effects of disease. Between-group differences in the hippocampal volumes, NAA /Cr, MI /Cr, NAA /MI ratios and hippocampal ADC were tested by t-tests. The level of significance was $p < 0.05$.

The sensitivity of MR measurements in distinguishing the three clinical groups (control, MCI, AD) were calculated at a fixed specificity of 80 %. Multi-variate logistic regression analysis was performed to assess whether combinations of the different MR measurements improved diagnostic discrimination among the three clinical groups. Stepwise modeling was used, stepping up, with $p < 0.05$ as the criterion for entry. Assessments for interaction and non-linearity (using polynomial regression) were made. The hierarchical principle was observed: main effects were not removed from the model if they appeared in higher order terms, still in the model. Age was included as an independent variable. A model with more than one MR term implied that the MR variables provided independent diagnostic information. Among the three metabolite ratio measurements (NAA /Cr, NAA /MI, and MI /Cr), the one ratio that discriminated with the highest sensitivity was chosen for each pair-wise discrimination analyses between the three clinical groups (control vs. MCI, control vs. AD, and AD vs. MCI). The chosen metabolite ratios were then compared to hippocampal W scores, hippocampal ADC and the multivariate model at a fixed specificity of 80% for discriminating among the three groups. Receiver operating characteristic (ROC) curves of hippocampal W scores, ADC, the appropriate single ^1H MRS measure, and the multi-variate model were plotted for each of these three pair-wise clinical group comparisons.

Results

Demographic aspects of the study group of 107 subjects (22 AD, 24 MCI and 61 controls) are presented in Table 1. The mean ages of the control, MCI and probable AD subjects were not different ($p > 0.05$). The differences in male/female ratios of the three groups were not statistically significant ($p > 0.05$). Education level was similar in all three groups ($p > 0.05$). The Mini- Mental State Examination (MMSE) scores of probable AD patients were lower than those of controls and patients with MCI ($p < 0.001$). The MMSE scores of patients with MCI were lower than that of control subjects ($p = 0.008$). One of oldest of the control subjects (93 years old) had an MMSE score of 22. Otherwise, the range of MMSE scores in controls were 25–30. The median CDR score in AD patients was one (range 0.5–2) corresponding to mild AD.

Age had no effect on NAA /Cr, MI /Cr, NAA /MI ratios and hippocampal ADC in the control subjects ($p>0.05$). There was however a negative correlation between age and normalized hippocampal volume (i.e. hippocampal volume / total intracranial volume). The hippocampal W scores we used for the analyses are by definition corrected (for age and gender) (10). The mean $\pm$ standard deviation (SD) of hippocampal volumes, posterior cingulate gyrus NAA /Cr, MI /Cr, NAA /MI ratios, and hippocampal ADC in control, MCI and AD subjects and the pair-wise between-group differences of the three clinical groups are presented in table 2. For almost all of the MR measurements, values obtained from the MCI patients were in between the AD and control subjects (table 2). The hippocampal volumes of AD and MCI patients were smaller than controls ($p<0.001$ for both). The hippocampal volumes AD patients were also smaller than patients with MCI were (nearly significant, $p=0.066$). Posterior cingulate gyrus NAA /Cr ratios were lower in AD patients than in controls ($p<0.001$), in AD patients than in patients with MCI ($p=0.004$), but not in MCI patients than in controls ($p>0.05$). Posterior cingulate gyrus MI /Cr ratios were higher in AD patients than in controls ($p<0.001$), in AD patients than in patients with MCI ($p=0.048$), and in MCI patients than in controls ($p=0.006$). Posterior cingulate gyrus NAA /MI ratios were lower in AD patients than in controls ($p<0.001$), in AD patients than in patients with MCI ($p=0.002$), and in MCI patients than in controls ($p=0.008$) (figure 2). The hippocampal ADC were higher in AD patients than in controls ($p=0.002$), and in MCI patients than in controls ($p=0.002$) There was no difference between the hippocampal ADC of MCI and AD patients ($p>0.05$)(table 2).

In logistic regression models, that were created for each inter-group comparison, the only measurements for distinguishing AD patients from MCI patients independently were NAA /Cr ($p=0.032$) and $(\text{NAA /Cr})^2$ ($p=0.040$). The measurements for distinguishing AD patients from normal elderly independently were, hippocampal volume ($p=0.021$) and a hippocampal volume - NAA /MI interaction ($p=0.050$). The measurements for distinguishing MCI patients from normal elderly independently were MI /Cr ($p=0.034$), hippocampal ADC ($p=0.027$), and hippocampal volume ($p=0.004$). Table 3 lists the final multi-variate model for each pair-wise inter-group discrimination.

The sensitivity of between-group discrimination with each measurement at a fixed specificity of 80% is listed in table 4. Among the three posterior cingulate gyrus ^1H MRS metabolite ratios, NAA /Cr had the highest sensitivity in distinguishing AD patients from patients with MCI (67%), MI /Cr ratios had the highest sensitivity in distinguishing MCI patients from controls (39%), and NAA /MI ratios had the highest sensitivity in distinguishing AD patients from controls (82%).

At a fixed specificity of 80 %, the sensitivity of distinguishing AD patients from patients with MCI improved to 72 % by using a quadratic model for NAA /Cr. The sensitivity for the multivariate model for distinguishing patients with MCI from controls was 75 %, and the sensitivity for the multivariate model was 86 % for distinguishing AD patients from controls (table 4). Figure 3 illustrates the ROC plots of inter-group discrimination involving hippocampal volumes, ADC, one of the ^1H MRS metabolite ratios that have the highest sensitivity at 80 % specificity for each inter-group discrimination (NAA /Cr for AD vs. MCI, MI /Cr for MCI vs. control, and NAA /MI for AD vs. control), and the multi-variate prediction model.

Discussion

Cognition exists along a continuum with normality and clear-cut AD at opposite ends of the spectrum. Every patient who receives a diagnosis of probable AD passes through a phase of mild cognitive impairment; that is, cognitive performance below what is considered normal for age, but insufficiently impaired to qualify for a clinical diagnosis of probable AD. It is

possible that every form of dementia will have its own clinically definable syndrome of mild cognitive impairment. For patients who lie along the continuum from normal cognition to AD, clinical diagnostic criteria for MCI (or amnesic MCI) have been established. These consist of an isolated memory deficit with preservation of cognitive performance in other domains. The concept of amnesic MCI has been embraced by many in aging and dementia research as useful. Individuals with this clinically definable syndrome are roughly ten-fold risk for progressing to AD within five years compared to normal elderly (3). Clinical trials are currently underway to determine if the risk of progressing from MCI to AD can be lowered. Diagnostic imaging tests, such as those evaluated in this paper, which address the diagnostic distinction between clinically identifiable groups (normal, MCI, and AD) may be useful. A consensus seems to be growing on the future importance of imaging as an aid in the early diagnosis of AD as well as in clinical trials. However, progress in this field has been hampered somewhat by the nature of the published literature. Most studies addressed a single imaging modality and often a single type of measurement from a given modality. Each research group naturally has evaluated diagnostic sensitivity and specificity in their own cohort of subjects. However, as our data demonstrate the efficacy of various MR tests differs as the composition of the clinical groups is changed. It has been difficult therefore to realistically compare the relative merits of different imaging techniques. The purpose of this study was to compare several MR techniques in the same group of subjects.

Discriminating MCI Patients from Normal Elderly

Hippocampal volume, ADC and ^1H MRS measurements obtained from MCI patients were between AD and control subjects in magnitude. This correlates well with clinical findings in patients with MCI whose cognitive performance lie between normal elderly and AD patients (32). Involvement of the entorhinal cortex and the hippocampus with neurofibrillary pathology, which coincides with neuron loss, has been demonstrated in pre-clinical AD and MCI patients (5–8). Our data show that, in MCI patients the hippocampus is atrophic and the diffusivity of water in the hippocampus is higher than in normal elderly subjects. A decrease in hippocampal volume is expected when there is neuron loss and atrophy. Increased water diffusivity in the hippocampi of MCI patients signifies a change in the microstructure of the hippocampus. This may be explained by loss of restrictive barriers like neuronal membranes and axonal processes, which would cause an expansion of the extracellular space, hence an increase in the diffusivity of water.

The ^1H MRS measurements revealed that MI /Cr is higher in the posterior cingulate gyri of patients with MCI than normal elderly. NAA /Cr levels from the same location while lower were not significantly different between MCI patients and controls. The metabolite NAA is exclusively present in neurons (33). Decrease in NAA levels in neurodegenerative diseases, has been attributed to neuronal loss or dysfunction (34–36). Reasons for the increase in the metabolite MI in AD are less clear (37). MI is primarily located in glial cells and is a possible glial marker (33). Therefore, elevation of MI in patients with MCI may be associated with glial activation and inflammation in the pathology of AD (38).

The logistic regression analysis revealed that the most sensitive discrimination of MCI patients from normal elderly was achieved with a multi -variate model that included hippocampal-W score, hippocampal ADC and posterior cingulate gyrus MI /Cr. However, most of the discriminatory power was carried by the hippocampal volume measurement. The addition of ADC and MRS to the model produced only a slight diagnostic improvement over the entire specificity range. However, the sensitivity of the multivariate model was considerably higher than the univariate hippocampal-W score at specificity of 85% and above (figure 3b).

Others have claimed that entorhinal cortex volumetry (39–41) and measurements of the entorhinal cortex length (42) are more accurate than hippocampal volumetry for early detection

of AD. In our hands, however, the accuracy of MR based volumetry of the hippocampus and entorhinal cortex is roughly equivalent in discriminating individuals with MCI and AD from normal elderly (43). The test-retest reproducibility of entorhinal cortex volumetry however, is lower than hippocampal volumetry, therefore we used hippocampal volumetry as the structural MR measurement in this study.

Discriminating patients with AD from normal elderly

In univariate analyses, the most sensitive measurements for distinguishing AD patients from elderly controls were hippocampal volumetry and NAA /MI. The sensitivity for the multivariate model was noticeably higher than any of the measurements individually at specificity of 90 % and above (figure 3a). Combining hippocampal NAA levels with hippocampal volume measurements in order to improve the separation of AD patients from normal elderly has been demonstrated in another study where NAA levels were measured from the hippocampi (19).

Discriminating patients with MCI from AD

The neuronal marker NAA /Cr levels from the posterior cingulate gyri discriminated patients with MCI from AD with a higher sensitivity than either hippocampal volumetry or hippocampal ADC. Early neurodegenerative pathology involves the anteromedial temporal lobes in patients with MCI (7). Therefore hippocampal measurements may not be as sensitive for discriminating MCI from AD as measurements from the posterior cingulate region which is involved later in the pathologic progression of AD. Our cross-sectional data cannot be directly extrapolated to address prediction of longitudinal clinical outcome. However, our data does suggest that it may be worthwhile to evaluate longitudinally whether posterior cingulate gyri NAA /Cr ratios serve as a marker for predicting or monitoring of progression from MCI to AD.

¹H MRS Measurements in discriminating among the clinical groups

Of the three ¹H MRS metabolite ratios, MI /Cr provided the highest sensitivity in discriminating MCI patients from normal. NAA /Cr provided the highest sensitivity in discriminating patients with MCI from AD, and NAA /MI, which is a combination of NAA /Cr and MI /Cr ratios provided the highest sensitivity in discriminating AD patients from normal. Based on these cross-sectional findings, it is reasonable to propose that, the initial ¹H MRS change in the in the pathologic progression of AD is an elevation in MI /Cr, which followed later by a decrease in NAA /Cr. If MI, which is primarily present in glial cells, is a marker for glial activation in AD; these results may imply that glial activation precedes neuronal loss or dysfunction in AD (38).

Limitations

Neurodegenerative changes involve the anteromedial temporal lobe earlier and more profoundly than other regions of the brain (13). We were unable to study the biochemical alterations in the anteromedial temporal lobe with single voxel ¹H MRS due to technical limitations (11). Alternatively, we were able to obtain reproducible, high quality spectra from the posterior cingulate gyrus, which is also part of the limbic cortex, albeit one involved later than the hippocampus in the progression of the disease (13). Another reason for studying posterior cingulate gyrus with single voxel ¹H MRS was that positron emission tomography has shown that glucose metabolism is significantly lower in the posterior cingulate gyri of people with AD and also in people who are at increased risk of developing AD than normal controls (44, (45). Had we been able to obtain high quality reproducible short TE MI /Cr ¹H MRS measurements from the hippocampus, the sensitivity of ¹H MRS in discriminating patients with MCI from normal elderly would likely have improved (19). This necessitates technical developments for better shimming of the anteromedial temporal lobe that is prone to

magnetic susceptibility artifacts, using smaller voxel size as in ^1H MRS imaging, and perhaps higher magnetic field strengths.

Although ADC was also measured from the hippocampi, sensitivity of ADC to discriminate among the three clinical groups was always less than hippocampal volumetry. This may be explained in part by lower spatial resolution of DWI compared to 3D SPGR images. We used an EPI-FLAIR pulse sequence to suppress the CSF signal in DWI (46). Therefore partial voluming of CSF would tend to depress the measured ADC of atrophic hippocampi, thus decrease the sensitivity of inter-group discrimination. We believe that the increased ADC measured in MCI and AD patients, is a true reflection of altered tissue microstructure, not a partial voluming artifact.

Another limitation of this study is the relatively small sample size in the MCI and AD groups, which particularly limited our ability to perform multivariate analyses. Thus, the absence of statistically significant associations cannot be definitively interpreted as implying that no association exists. In addition, estimates of sensitivity and specificity are based on the same data, which were used to identify the best predictor variables, so that these estimates may be somewhat optimistic. Prospective validation of our findings is needed. Furthermore, classification of the patients was based on clinical diagnosis. Assessing the diagnostic sensitivity and specificity of the multivariate model in absolute terms is only possible with histopathologic correlation. Correlating our findings with autopsy diagnosis will require many years.

Conclusion

The single MR measurement that best discriminated MCI patients from normal was hippocampal volumetry, discriminated MCI from AD was NAA /Cr ratios, and discriminated AD from normal was posterior cingulate gyrus NAA /MI or hippocampal volumetry. Measurement of several different phenomena (e.g. anatomy, biochemistry etc.) with MR within a single clinical examination is clinically practical. A multi-variate model improved diagnostic discrimination in all three pairwise inter-group comparisons overall, particularly at specificities approximately above 85 %. Our data suggests that a multi-modality (different MR variables) approach is slightly superior to any single MR test. Our data also indicate that the blend of MR tests that produce optimum diagnostic accuracy will differ as the disease severity progresses from normal to MCI to AD. The implication is that the search for a single “best imaging test” is probably misdirected. The specific MR measures used for clinical assessment and monitoring the efficacy of therapeutic intervention should be tailored to the clinical stage of the disease. MR measurements derived from the medial temporal lobe are likely to be more sensitive early in the disease progression, while measures derived elsewhere in the brain seem to be more sensitive at later stages. This matches the known pathologic progression of the disease. Prospective longitudinal studies on this cohort will assess the ability of multiple MR variables to predict future clinical course, which has practical implications both for diagnostic purposes and use of imaging as a surrogate endpoint in therapeutic trials.

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Figure 1.

Placement of the 8 cm³ ¹H MRS volume of interest (VOI) which is prescribed on a mid-sagittal T1 weighted image. The VOI is placed below the cingulate sulci and above the parietooccipital sulci, covering both of the posterior parts of posterior cingulate gyri and inferior precuneus.

Figure 2.

Examples of spectra obtained from the posterior cingulate gyri of an elderly control, a patient with MCI, and a patient with AD. MI /Cr peak area ratio is higher in the patient with MCI than the control and also higher in the AD patient than both the control and the patient with MCI. Posterior cingulate gyri NAA /MI peak area ratio is lower in the AD patient than the control and the patient with MCI.

Figure 3a:

Figure 3b:

Figure 3c: **Figure 3.**

Receiver operating characteristic (ROC) plots of inter-group discrimination for control vs. AD (a), control vs. MCI (b), and AD vs. MCI (c). Hippocampal volumes (line with squares), ADC (dotted line), one of the ^1H MRS metabolite ratios -NAA /Cr for AD vs. MCI, MI /Cr for MCI vs. control, and NAA /MI for AD vs. control (straight line), and all three measurements combined in a multi-variate model (line with circles) are presented.

Table 1

Demographic and clinical aspects of the subjects

	Control	MCI	AD
N	61	24	22
Age (mean $\pm$ SD)	80.6 $\pm$ 7.2	82.2 $\pm$ 5.1	79.2 $\pm$ 6.1
Male/Female	27 / 34	13 / 11	10 / 12
Education (median and range)	14 (7, 20)	13 (7, 20)	12 (8, 18)
MMSE (median and range)	29 (22, 30)	28 (19, 30)	20 (6, 27)

Table 2

MR measurements (mean $\pm$ SD) and pair wise between-group comparisons

MR measurement	Control n= 61	MCI n= 24	AD n= 22	Control vs. MCI	Control vs. AD	AD vs. MCI
Hippocampal W-scores	0.29 $\pm$ 1.24	-0.95 $\pm$ 1.34	-1.63 $\pm$ 1.09	P<0.001	P<0.001	P=0.066
Posterior cingulate gyrus NAA /Cr	1.52 $\pm$ 0.11	1.49 $\pm$ 0.09	1.40 $\pm$ 0.11	P=0.375	P<0.001	P=0.004
Posterior cingulate gyrus MI /Cr	0.62 $\pm$ 0.08	0.68 $\pm$ 0.08	0.73 $\pm$ 0.09	P=0.006	P<0.001	P=0.048
Posterior cingulate gyrus NAA /MI	2.48 $\pm$ 0.38	2.24 $\pm$ 0.28	1.97 $\pm$ 0.28	P=0.008	P<0.001	P=0.002
Hippocampal ADC*	1707 $\pm$ 83	1784 $\pm$ 131	1781 $\pm$ 116	P=0.002	P=0.002	P=0.937

*Right + Left hippocampal ADC

Table 3

Multi-variate prediction models for inter-group discrimination

Group	Variable	Beta	SE	P
Control vs. MCI	MI /Cr	8.2848	3.9156	0.034
	ADC	0.0070	0.0032	0.027
	HV ^I	-0.7264	0.2518	0.004
Control vs. AD	NAA /MI	-1.7100	1.4420	0.236
	HV	-6.6201	2.8732	0.021
	NAA /MI * HV	2.4129	1.2298	0.050
MCI vs. AD	NAA /Cr	-170.9	79.82	0.032
	NAA /Cr * NAA /Cr	54.52	26.49	0.040

^I HV: hippocampal volume

Table 4

Sensitivity of inter-group discrimination at a fixed specificity of 80%

		Control vs. MCI	Control vs. AD	MCI vs. AD
Univariate	Posterior cingulate gyrus NAA /Cr	17 %	61 %	67 %
	Posterior cingulate gyrus MI /Cr	39 %	68 %	44 %
	Posterior cingulate gyrus NAA /MI	38 %	82 %	45 %
	Hippocampal volumetry	79 %	86 %	45 %
	Hippocampal ADC	42 %	45 %	23 %
Multivariate		75 %	86 %	72 %