

The contribution of magnetic resonance spectroscopy as biomarker in Alzheimer's disease

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Abstract

The use of biomarkers is growing in the early detection of Alzheimer's disease (AD). Although some biomarkers such as medial temporal lobe volumetry, amyloid Positron Emission Tomography (PET), and A β 42 in CSF are being widely used, there is no clear consensus about the best biomarker to be used in each phase of the disease. Magnetic Resonance Spectroscopy (MRS) of the brain is less known as biomarker but has proven useful according to cross-sectional and longitudinal studies. This technique measures metabolite levels that reflect the degree of pathology in the brain. N-acetyl aspartate (NAA), a marker of neuronal density, decreases and Myo-inositol, a marker of glial proliferation, increases as the disease progresses. Decreased NAA levels have been detected in the prodromal phases of AD and even in presymptomatic stages in carriers of tau and amyloid protein mutations. Longitudinal studies have demonstrated good correlation between NAA levels and progression of AD, even in spite of treatment with cholinesterase inhibitors. From clinical trials we have learned that the current therapies have a modest effect on AD progression and that they do not have neuroprotective effects. This modest effect is reflected in the modest or null changes in metabolite levels in clinical trials using MRS as biomarker.

Key words

Magnetic resonance spectroscopy, Alzheimer's disease

1 Introduction

Given that Alzheimer's disease (AD) is a devastating and highly prevalent disorder in western countries, the use of biomarkers for early diagnosis is growing in the last decade. To date there are no sensitive enough and cost-efficient biomarkers to carry out in the general elderly population in the presymptomatic phases of the disease although this may change with the advances in this field. However the use of biomarkers has been recognized as useful in the condition that we call Mild Cognitive Impairment (MCI)^[1], especially in the amnesic form, which reaches a prevalence of around 6% in the elderly population^[2].

Amnesic MCI is defined as an elderly-related memory impairment that does not fulfill the criteria of dementia and that does not interfere with daily living activities. Of course other cognitive inefficiencies may be present. By abnormal memory in this context we mean a score in memory tests of 1.5 SD below that which is obtained in age and education-matched controls^[1]. The risk of conversion to dementia in MCI patients varies depending on conceptual definition and

follow-up period, between the following percentages: 12% per year for 4 years in comparison to 1%-2% annual for controls ^[1], 40% across 2 years ^[3], 34% over 4.5 years ^[4], 53% over 3 years ^[5], and 100% across 5-9.5 years ^[6, 7].

In the newly published criteria of AD a panel of experts established that an early diagnosis of AD can be made in amnesic MCI when clinical criteria are met plus the presence of one or two biomarkers ^[8]. They considered two types of biomarkers: those of neuronal injury (hippocampal volumetry, CSF tau, rate of brain atrophy, FDG PET, and SPECT), and those of amyloid deposition (CSF A β 42 and amyloid PET). A MCI syndrome is considered to be AD with high likelihood when both neuronal injury and amyloid deposition biomarkers are positive. When only one type of biomarker is positive then they consider an intermediate likelihood of being AD. Other neuroradiological techniques such as Diffusion MRI, perfusion MRI, and Magnetic Resonance Spectroscopy (MRS) are considered as less well validated biomarkers ^[8].

In this review we are focusing on MRS as biomarker of early AD.

2 Physical basis of MRS

Proton Magnetic Resonance Spectroscopy (MRS) allows us to investigate biochemical abnormalities in living tissues. The most frequently used spectroscopy is the technique that originates from the Hydrogen nuclei (proton 1H-MRS). This technique is based on the differences in resonance obtained from the hydrogen nuclei depending on the surrounding atoms (chemical shift) when a magnetic field is applied. Currently the spectra may be acquired with univoxel (SV) or multivoxel techniques (MV) ^[9]. The Univoxel technique has the advantages of better spatial location, greater homogeneity, better water suppression, and quicker acquisition of the spectrum as compared to the MV technique. However, only one spectrum can be obtained per acquisition. Conversely, the multivoxel technique (MV) makes it possible to obtain multiple spectra per acquisition simultaneously and to assess a greater area of the brain; however the spectral resolution is smaller. To date the uni-voxel technique is still superior to the MV technique on the grounds of reproducibility ^[10, 11].

Voxels must be positioned away from sources of susceptibility artifacts (air, fat, necrotic areas, cerebrospinal fluid, metal, calcification, and bone). For diffuse processes, a 2cm \times 2cm \times 2 cm voxel is routinely used. For local lesions, the SV can be reduced in volume ^[9].

The basic principle underlying single-voxel localization techniques is to use three mutually orthogonal slice selective pulses and design the pulse sequence to collect only the echo signal from the point (voxel) in space where all three slices intersect (see Figure 1). There are two modalities of spectral acquisition: PRESS or probe-p (point resolved spectroscopy) and STEAM (stimulated echo acquisition mode). The PRESS mode is used more frequently than STEAM mode because it increases the signal/noise ratio and is less sensitive to movement artifacts ^[12]. Each metabolite being assessed discloses a different frequency of hydrogen resonance and appears in a different site of the spectrum. The most frequently evaluated metabolites are N-acetyl-aspartate as neuronal and axonal marker (NAA), myo-inositol (mI) as glial marker, choline (Ch) that reflects the cellular membrane turnover, creatine (Cr) as marker of metabolism and internal reference value, and glutamate (Glx) as marker of metabolism of amino acids. The position of the metabolite signal is identified on the horizontal axis by its chemical shift, scaled in units referred to as parts per million (ppm). When all appropriate factors are considered, such as the number of protons and relaxation times a signal can be converted to a metabolite concentration by measuring the area under the curve. Given that water is the main component in living beings and its concentration is much higher than that of other metabolites, it is necessary to suppress the signal of resonance from the hydrogen of water. A plot showing peak amplitudes and frequencies is obtained. Each spectrum shows peaks corresponding to the different metabolite values: Myo-inositol (mI), 3.56 and 4.06 ppm; Choline compounds (Ch), 3.23 ppm; Creatine (Cr), 3.03 and 3.94 ppm; y N-acetyl-aspartate (NAA), 2.02; 2.5 and 2.6 ppm; Glx-glutamine and glutamate, 2.1-2.55 ppm and 3.8 ppm (see figure 2). The ratios between metabolites and creatine are also of great value as they counteract the systematic errors of measurements.

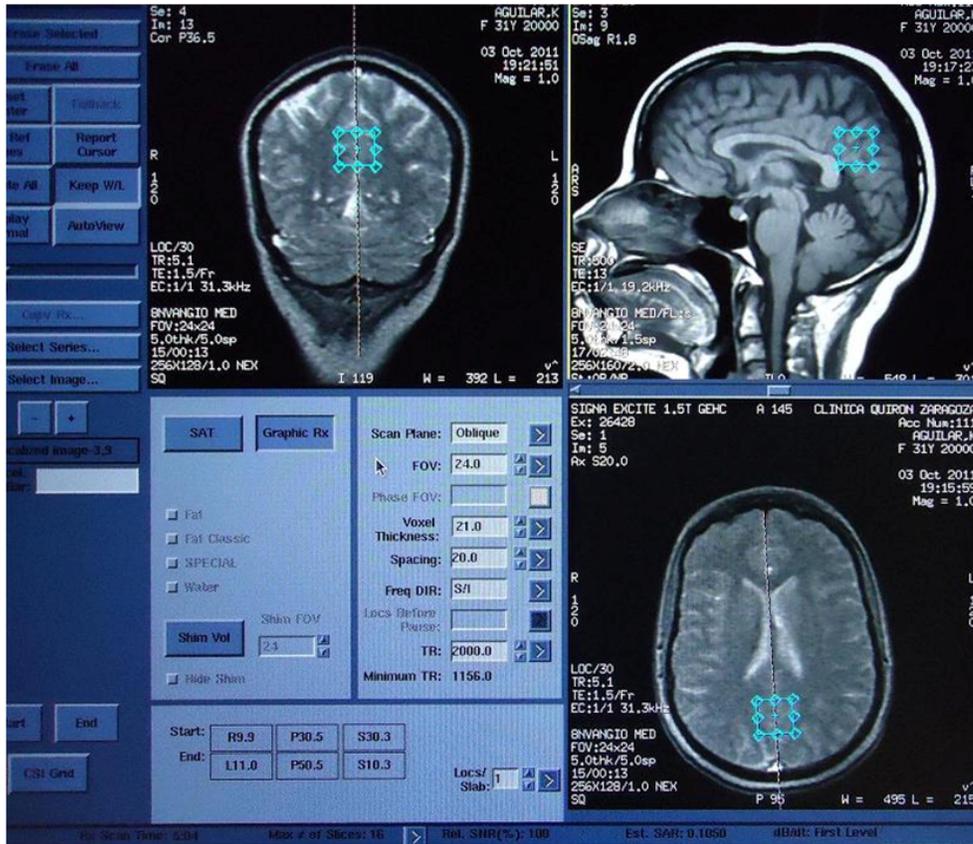


Figure 1. Positioning of a voxel in the bilateral posteromedial parietal cortex for study with frontal, sagittal and axial slices. The area explored includes the posterior cingulate gyrus and inferior precuneus.

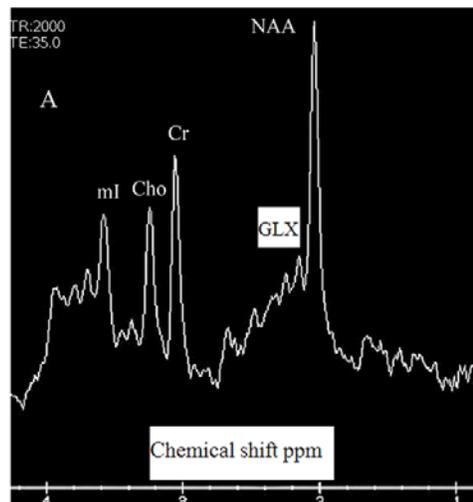


Figure 2. Example of spectrum in the same area with metabolite peaks.
 NAA: N-acetyl-aspartate; Ch. Choline compounds; Cr creatine, mI: myo-inositol. GLX: glutamate.

There is no consensus so far regarding the most appropriate Echo Times (TE) in MRS. A short TE (20-40 ms) allows us to increase the signal/noise ratio and to visualize most metabolite peaks, although it presents one inconvenience in that there

is of some degree of overlapping in the peaks. Intermediate TE (135-144 ms) inverts the lactate peak to better distinguish it from the lipids peak. Long TEs (270-288 ms) give a worse signal/noise ratio, but allow better visualization of some peaks (NAA, Choline, and Creatine), because they suppress the signal of others (myo-inositol, alanine, glutamate-glutamine) [13, 14].

3 MRS studies in Alzheimer's disease and MCI

There are cross-sectional studies dealing with MRS in AD. A study including 206 normal elderly subjects and 121 patients with Alzheimer's disease demonstrated a decrease in the NAA/Cr ratios as well as increased ml/Cr and Ch/Cr ratios in the left posterior cingulate gyrus in AD patients as compared with controls [15]. Alterations have been found in many cortical and subcortical areas; these have mainly been found in the medial temporal lobe [16, 17], but also in other cortical areas such as the frontal cortex [18, 19], the parietal [20, 21], and the occipital cortex [22-254]. Some studies suggest a continuum between normal aging, MCI and AD with regard to the values of N-acetyl aspartate in the brain [25, 26]. By means of RS of the posterior cingulate cortex, the NAA/Cr ratio discriminated MCI from AD patients at 67% sensitivity and 80% specificity [26].

Longitudinal studies also confirm the utility of MRS as biomarker. In a cohort of 53 MCI patients we found good predictive values with MRS with the voxel positioned in the medial left occipital lobe but not neither in the midparietal cortex nor in the hippocampus [27]. Theoretically the values in the hippocampus should have been better but this area is subject to artifacts due to the proximity of air and osseous structures. We repeated a similar study with a larger cohort of 71 MCI patients but on this occasion put the voxel in the left medial occipital lobe and in the bilateral posteromedial parietal cortex that included the posterior cingulate gyrus and inferior precuneus. The predictive values were better in the posteromedial parietal cortex (74% sensitivity; 83% specificity) but both areas were useful as target for prediction [28]. Other longitudinal studies with shorter follow-up periods yielded also good predictive values in the posteromedial parietal cortex [29], left paratrigenal area [30], and hippocampus [31].

Additional longitudinal studies showed valuable results with MRS. In a large cohort of 151 MCI patients (most being of amnesic 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 [32]. Longitudinal studies with repeated measurements are also of interest by demonstrating that NAA levels decrease in parallel with cognitive deterioration. 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 [33]. On the basis of another cohort composed of 16 MCI patients who converted to dementia and other 16 who did not, it was also observed that NAA levels decreased significantly more in converters than in non-converters [34].

The value of proton MRS as a biomarker was assessed ante-mortem in a single study with 54 patients ranging from low to high likelihood of having AD and who underwent autopsy. Decreases in NAA/Cr and increases in ml/Cr ratios correlated with higher Braak neuropathological stages in the posterior bilateral cingulate gyrus [35]. 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 [36] or protein tau [37] genes.

In comparison to other MRI techniques such as Diffusion and Perfusion MRI this technique is more objective and has proven more reliable [38].

Diffusion weighted imaging (DWI) is a structural method that assesses the microscopic translational movement of molecules via thermally driven random, so called Brownian motion of water molecules. The increased diffusion in brains with AD has been attributed to the decrease of neurons, axons and dendrites, leading to expansion of extracellular space

and spreading the water more quickly. However the value of diffusion MRI has been questioned as sensitivity is low to distinguish AD patients from controls using apparent diffusion coefficients in the hippocampus.

The available studies of Diffusion Tensor Imaging in mild AD are difficult to compare because a consensus on the best acquisition protocols, tensor metrics, and statistical analytical techniques has not yet emerged. Furthermore the pathophysiology of Diffusion Tensor Imaging alterations in AD has not been well defined.

Perfusion-MR is a functional technique that allows measurement of many hemodynamic parameters including relative cerebral blood volume, cerebral blood flow and mean transit time (MTT). Numerous techniques have been proposed in last years to measure various perfusion related parameters in the brain. Two approaches have proven successful: injection of paramagnetic contrast agents for measuring cerebral blood volumes (CBV) and arterial spin labeling (ASL) for measuring cerebral blood flow (CBF). ASL-MRI offers several advantages over PET and SPECT: a) it is free of exposure to ionizing radiation, intravenous contrast agents, and radioactive isotopes; b) it can be rapidly repeated because labeled water is cleared after a few seconds. An additional advantage is that perfusion and structural images can be acquired at the same imaging session. This technique is currently under investigation to try to improve the signal/noise ratio, and when achieved, it will probably be very effective and completely non-invasive^[38].

4 MRS to track progression of AD

As it was mentioned above the main findings of MRS in the brain of AD are decreased NAA levels and ratios and increased ml. A previous longitudinal study showed that NAA/Cr ratios declined significantly in MCI and AD patients, and it occurred in parallel with the progression of AD in the posterior cingulate gyrus despite 75% of AD patients took cholinesterase inhibitors. The ml/Cr ratios tended to increase but it was not significant. Surprisingly, the Ch/Cr ratios decreased in stable MCI patients compared with controls and MCI patients who convert to AD.³⁹ Several published trials measured the effect of drugs on AD progression with MRS^[40-47]. In table 1 are presented the main characteristics and outcomes of these trials. As we can see in general the drugs produced small changes in metabolite levels and ratios which correlated with the modest clinical or no effect of the drugs on AD progression. In a small trial with xanomeline, a muscarinic agonist, the cytosolic choline decreased in parietal cortex in comparison with controls.³⁹ The same authors tried to confirm the results in an additional trial but only 8 patients in total completed the trial.⁴¹ A randomized trial included 67 patients who were treated with either donepezil or placebo for one year. The NAA levels elevated transiently in the donepezil group at week 12 and 18 but the differences were not significant at endpoint, and cognitive improvement correlated with NAA elevations in the cortex. Conversely, in the placebo group the NAA concentration tended to remain near baseline values or to decrease modestly^[42]. In another controlled trial Rivastigmine tended to increase the NAA levels after 4 months in 24 AD patients in several areas of the brain with little improvement on cognition but without statistical significance in both variables, whereas in the comparison untreated group tended to decrease^[43]. A small and uncontrolled trial with 17 patients treated with donepezil for 4 months showed a correlation between cognitive function and NAA level changes in the parietal lobe.⁴⁴ In another study with 15 AD patients treated with donepezil the NAA levels in the right hippocampus decreased after 4 months with no changes in cognitive function^[45]. A randomized trial comparing donepezil with Memantine for 6 months showed no significant differences in clinical scales and metabolite levels. However in the whole sample of 63 patients the changes in the ADAS-cog correlated with the changes in the posteromedial parietal cortex^[46]. In a small double blind placebo controlled trial with Memantine the lack of changes in metabolite ratios correlated with the lack of clinical effect in AD compared with controls^[47].

In two of these trials it could be also concluded that the sample size required in a trial of AD patients to see relevant MRS changes was lower than the sample required if it is only based on the equivalent changes in clinical scales such as ADAS-cog^[43, 46].

Table 1. List of trials using MRS to monitor progression and response to treatment in AD.

AUTHOR	TYPE OF TRIAL	DRUG and follow-up	OUTCOMES
Satlin A et al 1997	Placebo Controlled. N=20	Xanomelina for 6 months	Decreased cytosolic choline
Krishnan 2003	RPCT. N=67	Donepezil for one year	Transient increased NAA/Cr in several areas
Modrego 2006	Controlled trial. N=34	Rivastigmine for 16 weeks	Increased NAA/Cr in anterior cingulate gyrus
Jessen 2006	Open trial. N=17	Donepezil for 3 months	Correlation clinical changes-NAA levels change in parietal lobe
Modrego 2010	Randomised comparative trial. N=63	Donepezil or Memantine for 6 months	No great differences in metabolite changes. Correlation clinical changes-MRS changes
Bartha 2008	Open. N=15	Donepezil for 4 months	NAA levels decreased in the hippocampus
Ashford 2011	DBPC trial. N=10	Memantine or placebo for 54 weeks	No significant changes. Correlation clinical changes-NAA/Cr changes

Note. RPCT: randomized placebo-controlled trial

A longitudinal study including 42 AD patients and 22 controls that underwent six Magnetic Resonance Spectroscopy studies within an elapse of time of 2 years showed that there is a progressive decline in the NAA/Cr ratios regardless of treatment with cholinesterase inhibitors ^[48].

Glutamate is another neurotransmitter studied with MRS. Increased glutamatergic excitotoxicity has been reported in AD but several cross-sectional reports showed decreased levels of glutamate in AD in comparison with controls ^[49-52]. In a small open trial, galantamine treatment for 4 months tended to elevate glutamate levels in the hippocampus ^[53].

5 Strengths and weaknesses of MRS to detect early AD

This technique has some advantages in comparison with other neuroradiological techniques. It is objective as metabolite measurements are made in an automated manner whereas medial temporal lobe structures volumetry is subject to inter-subject variability and artifacts ^[54]. In terms of reproducibility MRS reached a high intra-class correlation coefficient of metabolite levels, especially in the posteromedial parietal cortex, between two repeated measurements ^[55]. Furthermore the predictive values are similar to those obtained with volumetry in the two largest series ever studied in MCI: one of them included 389 patients) ^[56] in which sensitivity of whole brain volumetry was 75% and specificity was 68.8%; the other one ^[57] included 139 MCI patients who underwent hippocampal plus entorhinal volumetry, with sensitivity of 66.7% and specificity of 80%.

Of course there are some limitations to bear in mind with regard to MRS. Putting the voxel in the CSF or air may result in false metabolite measurements. Reliable values require the correction for atrophy and the presence of CSF with the appropriate and not always affordable software. Alternatively the use of the ratios to creatine can lessen this caveat. The homogeneity of the magnetic field should be checked frequently to avoid bad quality spectra. The voxel size should be appropriate for the structure examined. In this sense a voxel of 2cm×2 cm×2cm is too large for exploring the hippocampus but smaller areas can now be explored with modern scanners. To overcome these limitations the high-field 3T clinical scanners seem to be promising as they can discriminate between normality, MCI and AD in the hippocampus with small size voxels, although the number of patients was low ^[58, 59]. A detailed presentation of 1H MRS methodology developments and subsequent quantitation has been published ^[60, 61].

So far MRS has proven useful in MCI and AD but we do not have enough studies to advocate use in presymptomatic stages of AD. For this purpose other biomarkers are better known (CSF Aβ42 and amyloid PET) ^[62]. What we call

amnesic MCI may be considered as a prodromos of AD or a condition at high risk of conversion to AD because eventually most patients convert to dementia. There are two reports in which over time 100% of patients had converted to dementia when followed for 10 years^[6,7]. In a retrospective study we saw that AD patients preceded by MCI for longer than one year periods had lower NAA levels in several areas of the brain and more depressive symptoms that those not preceded by evident prodromal MCI^[63]. Therefore the studies conducted in amnesic MCI are biased in the sense of an excess of conversion rates and we should use the expression “early conversion to AD” rather than conversion to AD.

In conclusion, MRS is a useful tool in AD not only for early detection but also for tracking progression of the disease with and without pharmacological treatment. Studies comparing this technique with other biomarkers are warranted.

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