Utility of SPM8 plus DARTEL (VSRAD) Combined with Magnetic Resonance Spectroscopy as Adjunct Techniques for Screening and Predicting Dementia due to Alzheimer’s Disease in Clinical Practice

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Abstract We validated the utility of SPM8 plus DARTEL (VSRAD) combined with magnetic resonance spectroscopy (¹H MRS) as an adjunct screening technique for dementia due to Alzheimer’s disease (AD). We examined the posterior cingulate gyri of 228 subjects using VSRAD and ¹H MRS in addition to conventional cerebrospinal fluid biomarkers at baseline. At the 3-year follow-up, the 228 subjects were classified as follows: 93 healthy subjects, 42 MCI-non-converters (MCI-NC), 25 MCI-converters to AD (MCI-C), 44 AD, 8 dementia with Lewy bodies (DLB), 5 normal pressure hydrocephalus, and 11 patients with other neurological diseases. Our results demonstrated that subjects with increased medial temporal atrophy (MTA) severity on VSRAD, increased Cho/Cr, MI/Cr ratio, and decreased NAA/Cr and NAA/MI ratio on ¹H MRS at baseline were at risk of dementia due to AD. Receiver operating characteristic analysis showed that severity of MTA and the NAA/MI ratio distinguished patients with AD and MCI-C from controls. Furthermore, the 118 subjects without dementia and MTA showing only a decreased NAA/MI ratio at baseline developed to MCI-C, AD, and DLB 3 years later. ¹H MRS detected biochemical abnormalities preceding brain atrophy and cognitive decline. VSRAD combined with ¹H MRS may be routinely applied to screen for MCI/AD and prodromal AD in clinical practice.

Keywords: Alzheimer’s disease, magnetic resonance imaging, magnetic resonance spectroscopy (¹H MRS), screening, SPM8 plus DARTEL (VSRAD), surrogate marker

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The CSF biomarkers: amyloid-beta for identifying subjects at risk for AD is important. The preclinical stage of MCI/AD, establishing a system for screening and preventive intervention from the early diagnosis of AD begins about 20 years before the clinical onset of mild cognitive impairment (MCI) and AD [1, 2]. Thus, it is considered that treatments using disease-modifying drugs after onset of AD may be too late, and screenings and preventive intervention from the preclinical stage could be an efficient strategy for overcoming AD. For the purpose of preventive therapy at the preclinical stage of MCI/AD, establishing a system for identifying subjects at risk for AD is important. The CSF biomarkers: amyloid-beta (Aβ42, Aβ40), total tau (t-tau), and phosphorylated tau (p-tau) obtained by lumbar puncture have been reliable biomarkers reflecting the underlying pathogenesis of AD; however, obtaining them is invasive for patients [3–7]. Development of non-invasive neuroimaging techniques such as amyloid-PET and structural MRI including voxel-based morphometric MRI (VBM-MRI) facilitate early accurate diagnosis and help explain the underlying pathogenesis of MCI, AD, and dementia with Lewy bodies (DLB) [8–15]. Very recently, the development of tau-PET, which allows the visualization of tau deposition directly in the brain, while exciting [16–18], will take a long time to be applied in our memory clinic.

The screening methods for capturing subjects at risk for early MCI/AD or preclinical AD should be popular, easy to handle, non-invasive, widely distributed, and costless. Although the amyloid and tau imaging methods enable direct observation of AD pathogenesis, they are not appropriate methods for easily screening dementia in most hospitals and are still not easily applied in clinical practice. In that regard, VBM-MRI that could detect AD-specific structural brain changes, especially medial temporal atrophy (MTA) [8–11], and magnetic resonance spectroscopy (1H MRS) using regular 1.5-Tesla (T) MRI that captures early brain biochemical abnormalities, are popular, non-invasive, cost-effective, require a short examination time, and are practical for early diagnosis and for tracking subjects with dementia due to AD [19–32]. Previous studies have shown the utility of VBM-MRI for showing MTA and metabolite abnormalities on 1H MRS for the early diagnosis of MCI and AD [8–11, 19–33]. The aim of our study was to validate the utility of SPM8 plus diffeomorphic anatomic registration through an exponentiated lie algebra (DARTEL) and voxel-based specific regional analysis system for Alzheimer’s disease (VSRAD), combined with 1H MRS relevant CSF biomarkers (Aβ42, p-tau, and Aβ40/p-tau) as adjunct early screening methods for dementia due to AD in clinical practice.

INTRODUCTION

Many clinical studies, including the Alzheimer’s Disease Neuroimaging Initiative and Dominantly Inherited Alzheimer Network, using Alzheimer’s disease (AD) related biomarkers such as cerebrospinal fluid (CSF) biomarkers, structural magnetic resonance imaging (MRI), and amyloid positron emission tomography (PET), suggest that the underlying pathology of AD begins about 20 years before the clinical onset of mild cognitive impairment (MCI) and AD [1, 2]. For the purpose of preventive therapy at the preclinical stage of MCI/AD, establishing a system for identifying subjects at risk for AD is important. The CSF biomarkers: amyloid-beta (Aβ42, Aβ40), total tau (t-tau), and phosphorylated tau (p-tau) obtained by lumbar puncture have been reliable biomarkers reflecting the underlying pathogenesis of AD; however, obtaining them is invasive for patients [3–7]. Development of non-invasive neuroimaging techniques such as amyloid-PET and structural MRI including voxel-based morphometric MRI (VBM-MRI) facilitate early accurate diagnosis and help explain the underlying pathogenesis of MCI, AD, and dementia with Lewy bodies (DLB) [8–15]. Very recently, the development of tau-PET, which allows the visualization of tau deposition directly in the brain, while exciting [16–18], will take a long time to be applied in our memory clinic.

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MATERIALS AND METHODS

Demographic characteristics of the subjects

In total, 228 subjects underwent VBM-MRI and 1H MRS at the same time as lumbar puncture for the analysis of CSF biomarkers (Aβ42 and p-tau) at baseline. All subjects were recruited from the memory clinic of Higashi Matsudo Municipal Hospital between September 2010 and October 2013. The subjects suspected of having vascular dementia, mixed dementia, other degenerative diseases, and hereditary dementia were excluded from this study. None of the subjects was taking acetylcholinesterase inhibitors at baseline. Informed consent was provided by each participant or his/her relative. Our protocol was approved by the Ethics Committees of the Higashi Matsudo Municipal Hospital. Some of the subjects underwent MR/MRS examinations serially from 2010 to 2013, equaling 1,644 times in total. All subjects were followed-up for at least 3 years. After at least 3 years of follow-up from baseline, the subjects were accurately diagnosed and classified into six groups: non-demented healthy controls; amnestic MCI-non-converters (MCI-NC), who remained stable from baseline; MCI-converters (MCI-C), who converted from amnestic MCI to AD within 3 years from baseline; AD based on the clinical diagnosis of probable AD according to the diagnosis of dementia due to AD: Recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease; DLB; normal pressure hydrocephalus (NPH); and non-demented other neurological diseases (OND), consisting of four cases of viral meningitis, five patients with peripheral neuropathy, and two patients with amyotrophic lateral sclerosis (ALS). The subjects were diagnosed based on clinical criteria [34–38]. In addition to these clinical investigations, the diagnosis of DLB was confirmed based on the results of 123I-MIBG myocardial scintigraphy showing that myocardial 123I-MIBG uptake was impaired in early-stage DLB [39].

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All CSF samples were obtained by lumbar puncture between 2 P.M. and 3 P.M. and were collected into polypropylene tubes. The CSF samples were centrifuged (1,000 × g for 10 min at 4°C) to remove any debris and then stored in small aliquots at −80°C.

The MRI and lumbar puncture were performed within a gap of 2 days. CSF Aβ40 and Aβ42 were measured by enzyme-linked immunosorbent assay (ELISA) using a Human Beta Amyloid (1–40) ELISA Kit (Wako, 292-62301; Wako Chemical Co.) and a Human Beta Amyloid (1–42) ELISA Kit (Wako, 298-62401; Wako Chemical Co.), respectively. CSF p-tau proteins were measured using INNOTEST® PHOSPHO-TAU (181P; Innogenetics, Belgium).

SPM8 plus DARTEL (VSRAD) and ¹H MRS

MRI and single voxel (SV) ¹H MRS examinations were performed using a Vantage Titan 1.5-T scanner (TOSHIBA, Tochigi, Japan) (T1, T2, and fluid attenuated inversion recovery [FLAIR] MRI) with a 14-channel phased array coil. Total imaging time of MRI including VSRAD and ¹H MRS was 30 min.

SPM8 plus DARTEL (VSRAD)

For SPM8 plus DARTEL (VSRAD), we acquired 110 three-dimensional (3D) sections (1.5-mm thick) of a T1-weighted magnetization-prepared rapid acquisition gradient echo sequence in a sagittal orientation (field of view [FOV] = 23, repetition time [TR] = 9.7 ms, echo time [TE] = 5.5 ms, flip angle = 20°, and inversion time [TI] = 300 ms, with no intersection gaps).

The degree of brain atrophy within the target volume of interest (VOI) of the grey matter in the medial temporal lobe (MTL) was determined using automatic VBM-MRI by SPM8 plus DARTEL called as VSRAD developed by Matsuda et al. [11]. The intensity of the VOI was evaluated by Z-score = (control mean – individual value)/(control SD). These Z-score maps were displayed by overlay on tomographic sections and surface renderings of the standardized brain.

¹H MRS

A 3D high-resolution gradient echo (3D-GRE) acquisition with TR/TE = 15/5.5 ms, flip angle of 20 degrees, in-plane resolution of 1.5 mm, and a slice thickness of 1.5 mm was performed for anatomic segmentation. ¹H MRS studies were performed using the automated MRS package Proton Brain Examination (PROBE)/SV. A point resolved spectroscopy sequence with TR = 2,000 ms, TE = 25 ms, 2,048 data points, and 128 excitations was used for the examinations. An 8-cm³ (2 × 2 × 2 cm³) ¹H MRS voxel was prescribed on a mid sagittal 3D-GRE image, including the right and left posterior cingulate gyrus (PCG) (Fig. 1). After analyzing each PROBE/SV acquisition, metabolite intensity ratios were calculated. Quantifying metabolite intensities with an internal standard as a reference is preferred in clinical ¹H MRS because internal referencing does not require correction for coil loading, atrophy, and relaxation times and can readily be used in clinical practice with standard equipment and vendor-provided processing software. The PCG of both hemispheres were selected as VOI of ¹H MRS because ¹H MRS of the PCG is technically stable and it is possible to obtain information in a short time (4 min) without noise in our 1.5-T MRI. In addition, previous studies showed that the PCG is a well-defined midline structure from which reproducible, high quality, single-voxel spectra at regular 1.5-T MRI can be acquired compared to the hippocampus, which is anatomically small and sometimes difficult to analyze because of CSF contamination by the inferior horn of the lateral ventricle. The PCG also has significant metabolite measurement reliability and higher sensitivity to early AD pathology compared with other regions of the brain including the hippocampus [20, 40, 41]. The peak height ratios of NAA, choline (Cho), and MI normalized to Cr levels (NAA/Cr, Cho/Cr, MI/Cr, and NAA/MI) were evaluated in this study [19–32, 40–45].

Statistics

Statistical calculations were performed using GraphPad Prism® software (GraphPad Software). Values are indicated as the mean ± the standard deviation (SD) and the median ± the interquartile deviation. Groups were compared using χ² tests (two groups) or Kruskal–Wallis tests (three and four groups). Correlations between each factor were examined using Spearman’s rank correlation analyses. A linear model was applied to the data to obtain the correlation coefficient (rs) and p value.

RESULTS

The demographic characteristics of the patients are listed in Table 1. After at least 3 years of follow-up...
Fig. 1. Pictures of sagittal and axial MRIs showing PCG defined as a voxel of interest (VOI) of 1H MRS examination in this study (upper picture). The left lower picture shows the analyzed peak of each metabolite. The right lower table shows the measured peak height of each metabolite (NAA, Cr, Cho, and MI) using 1H MRS in the PCG. We used the NAA/Cr, Cho/Cr, MI/Cr, and NAA/MI ratios as described in the Materials and Methods.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>MCI-NC</th>
<th>MCI-C</th>
<th>AD</th>
<th>DLB</th>
<th>NPH</th>
<th>OND</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>93</td>
<td>42</td>
<td>25</td>
<td>44</td>
<td>8</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Age (±SD)</td>
<td>74.6 (±10.2)</td>
<td>78.9 (±7.5)</td>
<td>77.4 (±7.1)</td>
<td>80.6 (±7.3)</td>
<td>76.0 (±5.3)</td>
<td>76.0 (±4.2)</td>
<td>74.0 (±5.3)</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>54/39</td>
<td>23/19</td>
<td>18/7</td>
<td>26/18</td>
<td>3/5</td>
<td>3/2</td>
<td>4/7</td>
</tr>
<tr>
<td>CDR sum of boxes</td>
<td>0</td>
<td>2.0 ±0.5</td>
<td>2.5 ±1.0</td>
<td>3.5 ±1.0</td>
<td>4.5 ±0.5</td>
<td>3.5 ±0.5</td>
<td>3.5 ±0.5</td>
</tr>
<tr>
<td>MMSE (±SD)</td>
<td>27.2 (±2.8)</td>
<td>25.5 (±2.5)</td>
<td>24.1 (±1.8)</td>
<td>20.7 (±3.2)</td>
<td>17.3 (±6.0)</td>
<td>19.6 (±4.6)</td>
<td>27.5 (±2.1)</td>
</tr>
<tr>
<td>APOE4 allele</td>
<td>27.2%</td>
<td>47.0%</td>
<td>54.5%</td>
<td>40.9%</td>
<td>90.0%</td>
<td>33.0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Demographic and clinical data of the 228 subjects (control: 93, MCI-NC: 42, MCI-C: 25, AD: 44, DLB: 8, NPH: 5, OND: 11) diagnosed based on clinical criteria after 3 years of follow-up from baseline. After 3 years of follow-up observations, the 228 subjects were diagnosed as healthy controls, MCI-NC, MCI-C, AD, DLB, NPH, and OND, AD, Alzheimer’s disease; CDR, Clinical Dementia Rating; DLB, dementia with Lewy bodies; MCI-NC, mild-cognitive impairment non-converter (stable MCI); MCI-C, MCI-converted to AD; MMSE, Mini-Mental State Examination; NPH, normal pressure hydrocephalus; OND, other neurological diseases consisting of four cases of viral meningitis, five patients with peripheral neuropathy, and two patients with amyotrophic lateral sclerosis (ALS).

CSF biomarkers at baseline

The levels of CSF Aβ42, p-tau, and p-tau/Aβ42 at baseline are listed in Supplementary Table 1. Details of individual subjects are shown in Supplementary Figure 1a and b.

Receiver operating characteristic (ROC) analysis showed that the level of CSF Aβ42 could differentiate from baseline, the 228 subjects were diagnosed based on clinical criteria [34–38], and were classified into six groups: healthy controls (n=93); MCI-NC (n=42), who had stable MCI from baseline; MCI-C (n=25), who converted from MCI to AD within 3 years from baseline; AD (n=44); DLB (n=8); NPH (n=5); and OND (n=11), as described in the Materials and Methods.
Fig. 2. a) Results of the ANOVA of SPM8 plus DARTEL (VSRAD) at baseline showed VSRAD is useful for diagnosing MCI-NC, MCI-C, and AD from controls. MTA, Medial temporal lobe atrophy. b) ROC analysis of VSRAD for distinguishing MCI-C and AD from healthy controls using the severity of MTA: Z-score and extent of grey matter atrophy (%). ROC analysis of the severity of MTA on VSRAD showed an AUC of 0.90, sensitivity 84.1%, and specificity 84.6% at the cut off Z-score of 1.75 in controls versus AD, while controls versus MCI-C showed an AUC of 0.86, sensitivity 85.7%, and specificity 80.8% at the cut off Z-score of 1.54.

AD from controls with an area under the curve (AUC) of 0.92, sensitivity 88.3%, and specificity 76.0% at a cut off value of 625.1 pg/mL. Furthermore, the level of CSF Aβ42 that differentiated MCI-C from controls had an AUC of 0.80 with sensitivity 73.7%, and specificity 76.0% at a cut off value of 495.9 pg/mL. The CSF p-tau could differentiate AD from controls (AUC = 0.78) with sensitivity 73.1%, and specificity 76.0% at a cut off value of 41.9 pg/mL, and could differentiate MCI-C from controls with sensitivity 73.7% and specificity 76.0% at a cut off value of 40.9 pg/mL. The CSF p-tau/Aβ42 ratio could differentiate AD from controls (AUC = 0.96) with sensitivity 92.3% and specificity 92.0% at a cut off value of 0.08, and could differentiate MCI-C from controls (AUC = 0.85) with sensitivity 79.0% and specificity 80.0% at a cut off value of 0.06. The CSF p-tau and the CSF p-tau/Aβ42 ratio at baseline could significantly distinguish MCI-NC, MCI-C, and AD patients from healthy controls (p < 0.0001) (Fig. 2a). The Z-score showing the severity of the MTA was 1.25 (mean) ± 0.71 (SD) [median, 1.10], 2.39 ± 1.00 [2.20], 2.55 ± 1.13 [2.25], 2.77 ± 1.11 [2.45], 1.92 ± 1.02 [1.64], 1.71 ± 0.24 [1.69], 1.99 ± 1.11 [1.71] in controls, MCI-NC, MCI-C, AD, DLB, NPH, and OND, respectively (Table 2).

ROC analysis of the severity of MTA on VSRAD showed an AUC of 0.90, sensitivity 84.1%, and specificity 84.6% at the cut off Z-score of 1.75 in controls versus AD, while controls versus MCI-C showed an AUC of 0.92, sensitivity 82.3%, and specificity 80.8% at the cut off Z-score of 1.54 (Fig. 2b).
Results of SPM8 plus DARTEL (VSRAD) and $^1$H MRS in the posterior cingulate gyrus (PCG) at baseline. Data shown are the mean ± SD and (median). The severity of MTA on VSRAD increased in MCI and AD at baseline. NAA/Cr and NAA/MI ratios decreased in MCI-C, AD, and DLB at baseline. The referential combined value of VSTAD and $^1$H MRS obtained using the following equation: severity of MTA = Z-score/[NAA/MI]

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MCI-NC</th>
<th>MCI-C</th>
<th>AD</th>
<th>DLB</th>
<th>NPH</th>
<th>OND</th>
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<tbody>
<tr>
<td>n</td>
<td>a=93</td>
<td>a=42</td>
<td>a=25</td>
<td>a=44</td>
<td>a=8</td>
<td>a=5</td>
<td>a=11</td>
</tr>
<tr>
<td>Severity of MTA: Z-score</td>
<td>1.25 ± 0.71</td>
<td>2.39 ± 1.00</td>
<td>2.55 ± 1.13</td>
<td>2.77 ± 1.14</td>
<td>1.92 ± 1.02</td>
<td>1.71 ± 0.24</td>
<td>1.99 ± 1.11</td>
</tr>
<tr>
<td>mean ± SD (median)</td>
<td>(1.10)</td>
<td>(2.14)</td>
<td>(2.25)</td>
<td>(2.45)</td>
<td>(1.64)</td>
<td>(1.69)</td>
<td>(1.71)</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>1.74 ± 0.11</td>
<td>1.66 ± 0.10</td>
<td>1.58 ± 0.13</td>
<td>1.57 ± 0.13</td>
<td>1.73 ± 0.15</td>
<td>1.77 ± 0.13</td>
<td>1.77 ± 0.13</td>
</tr>
<tr>
<td>mean ± SD (median)</td>
<td>(1.74)</td>
<td>(1.65)</td>
<td>(1.64)</td>
<td>(1.59)</td>
<td>(1.59)</td>
<td>(1.66)</td>
<td>(1.78)</td>
</tr>
<tr>
<td>NAA/MI</td>
<td>1.92 ± 0.26</td>
<td>1.8 ± 0.25</td>
<td>1.66 ± 0.21</td>
<td>1.72 ± 0.46</td>
<td>1.52 ± 0.22</td>
<td>1.96 ± 0.43</td>
<td>1.89 ± 0.21</td>
</tr>
<tr>
<td>mean ± SD (median)</td>
<td>(1.90)</td>
<td>(1.79)</td>
<td>(1.65)</td>
<td>(1.62)</td>
<td>(1.58)</td>
<td>(1.91)</td>
<td>(1.89)</td>
</tr>
<tr>
<td>Severity of MTA: Z-score/[NAA/MI]</td>
<td>1.24 ± 0.72</td>
<td>2.34 ± 1.00</td>
<td>2.55 ± 1.13</td>
<td>2.81 ± 1.13</td>
<td>1.87 ± 0.98</td>
<td>1.71 ± 0.24</td>
<td>2.07 ± 1.22</td>
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<tr>
<td>mean ± SD (median)</td>
<td>(1.07)</td>
<td>(2.14)</td>
<td>(2.25)</td>
<td>(2.56)</td>
<td>(1.64)</td>
<td>(1.69)</td>
<td>(1.71)</td>
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**Results of $^1$H MRS in the PCG at baseline**

The ratio of NAA/Cho was significantly decreased in the following order compared to controls: AD ($p=0.0000$), MCI-C ($p=0.0001$), and MCI-NC ($p=0.0007$), and DLB ($p=0.0027$) (Fig. 3a). The ratio of Cho/Cr was not significantly different among the controls, MCI-NC, MCI-C, AD, DLB, NPH, and OND patients (data not shown). The ratio of Cho/Cr increased in MCI-C ($p=0.009$), AD ($p=0.0359$), and DLB ($p=0.0009$) patients compared to controls. There was also a
Fig. 4. Representative case with no apparent dementia and MTA showing abnormalities of $^1$H MRS and CSF biomarkers that converted to MCI due to AD 3 years later. A 70-year-old woman with MMSE: 26 and MTA (Z-score: 0.59); however, $^1$H MRS in the PCG showed an increased MI/Cr ratio and decreased NAA/Cr and NAA/MI ratios, while CSF biomarkers showed decreased Aβ42 and increased p-tau in September 2010. She developed to MCI/AD with MMSE: 21, and VSRAD showed apparent MTA (Z-score: 1.65) in October 2013. Arrows: ↑ increase, ↓ decrease.

In the ROC analysis of the $^1$H MRS in the PCG, the ratio of NAA/MI demonstrated an AUC of 0.75, sensitivity 71.4%, and specificity 71.2% at the cut off value of 1.77 in controls versus AD, and an AUC of 0.77, sensitivity 77.5%, and specificity 70.2% at the cut off value of 1.79 in controls versus MCI-C. Our results showed the ratios of NAA/Cr and NAA/MI of the $^1$H MRS in the PCG could be useful for differentiating AD, MCI-C due to AD, and DLB from healthy controls (Fig. 3d).

Utility of $^1$H MRS in subjects without apparent MTA (severity of MTA: Z-score ≤3.5) at baseline on VSRAD

Figure 4 shows a representative case of a 70-year-old woman presenting with very mild cognitive impairment and a Mini-Mental State Examination (MMSE) score of 26 in September 2010. The results of this case suggest the utility of $^1$H MRS without apparent difference between MCI-NC and MCI-C patients ($p=0.0022$; Fig. 3b). The ratio of NAA/MI was significantly decreased in the following order compared to controls: DLB (1.52 ± 0.22 [1.58], $p=0.0000$), AD (1.72 ± 0.48 [1.62], $p=0.0000$), MCI-C (1.66 ± 0.21 [1.65], $p=0.0000$), and MCI-NC (1.82 ± 0.25 [1.79], $p=0.0000$). There was a difference between MCI-NC and MCI-C patients ($p=0.0005$), as well as in the MI/Cr ratio (Fig. 3c). These results may suggest that decreased NAA/Cr and NAA/MI, and increased MI/Cr in the PCG may correspond to neuronal injury, which reflects a decrease in NAA and gliosis, which reflects an increase of MI in the progression of the underlying pathology of AD and DLB in the brain.
Table 3

<table>
<thead>
<tr>
<th>n = 118</th>
<th>Control n=79</th>
<th>MCI-NC n=7</th>
<th>MCI-C n=11</th>
<th>AD n=6</th>
<th>DLB n=6</th>
<th>NPH n=8</th>
<th>OND n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VSRAD Severity of MTA; Z-score</strong></td>
<td>0.94±0.32</td>
<td>1.22±0.42</td>
<td>1.24±0.30</td>
<td>1.28±0.15</td>
<td>1.19±0.25</td>
<td>1.46±1.00</td>
<td>1.10±0.30</td>
</tr>
<tr>
<td><strong>mean ± SD (median)</strong></td>
<td>(0.92)</td>
<td>(1.23)</td>
<td>(1.22)</td>
<td>(1.28)</td>
<td>(1.21)</td>
<td>(1.14)</td>
<td></td>
</tr>
<tr>
<td><strong>1H MRS NAA/Cr</strong></td>
<td>1.74±0.11</td>
<td>1.65±0.10</td>
<td>1.65±0.10</td>
<td>1.54±0.01</td>
<td>1.66±0.15</td>
<td>1.64±1.01</td>
<td>1.71±0.14</td>
</tr>
<tr>
<td><strong>mean ± SD (median)</strong></td>
<td>(1.74)</td>
<td>(1.63)</td>
<td>(1.62)</td>
<td>(1.52)</td>
<td>(1.50)</td>
<td>(1.72)</td>
<td></td>
</tr>
<tr>
<td><strong>3H MRS NAA/AI</strong></td>
<td>1.91±0.26</td>
<td>1.91±0.23</td>
<td>1.60±0.13</td>
<td>1.31±0.09</td>
<td>1.43±0.27</td>
<td>1.38±1.57</td>
<td>1.87±0.25</td>
</tr>
<tr>
<td><strong>mean ± SD (median)</strong></td>
<td>(1.90)</td>
<td>(1.91)</td>
<td>(1.64)</td>
<td>(1.50)</td>
<td>(1.55)</td>
<td>(1.82)</td>
<td></td>
</tr>
<tr>
<td><strong>Change of MMSE</strong></td>
<td>27.8±2.6</td>
<td>26.0±1.5</td>
<td>25.0±1.3</td>
<td>22.7±2.3</td>
<td>17.3±4.0</td>
<td>19.6±2.0</td>
<td>27.5±2.1</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td>(27.0)</td>
<td>(26.2)</td>
<td>(25.4)</td>
<td>(23.2)</td>
<td>(17.0)</td>
<td>(27.3)</td>
<td>(27.3)</td>
</tr>
<tr>
<td><strong>3 years later from baseline</strong></td>
<td>27.6±2.5</td>
<td>24.5±1.6</td>
<td>22.6±1.5</td>
<td>20.2±1.5</td>
<td>15.9±1.2</td>
<td>26.2±7.1</td>
<td>27.1±1.5</td>
</tr>
</tbody>
</table>

The NAA/Cr was 1.74±0.11 [1.74] in controls, 1.65±0.10 [1.63] in MCI-NC, 1.65±0.10 [1.62] in MCI-C, 1.54±0.08 [1.52] in AD, 1.46±0.15 [1.50] in DLB, 1.64 in NPH, and 1.71±0.14 [1.72] in OND patients. The NAA/AI was 1.91±0.26 [1.90] in controls, 1.91±0.23 [1.93] in MCI-NC, 1.68±0.17 [1.68] in MCI-C, 1.51±0.09 [1.50] in AD, 1.43±0.27 [1.55] in DLB, 1.38 in NPH, and 1.87±0.25 [1.82] in OND patients. The changes in MMSE scores from baseline to 3 years later were as follows: 27.8±2.6 (27.0) to 27.0±2.5 (27.0) in controls, 26.0±1.5 (26.2) to 24.5±2.5 (26.0) in MCI-NC, 25.0±1.3 (25.4) to 22.6±1.5 (26.0) in MCI-C, 22.7±4.0 (17.0) to 20.2±1.5 (20.6) in AD, 19.6±2.0 in NPH, and 27.5±1.5 (27.1) in OND patients (Table 3).

The value of the Z-score (severity of MTA/NAA/AI) as a referential indicator of a combined VSRAD and 1H MRS marker

We tried to create an indicator that would estimate the utility of VSRAD combined with 1H MRS as adjunct screening techniques for dementia due to AD. The Z-score value calculated as: severity of MTA on VSRAD/(NAA/AI) on 1H MRS was chosen as a referential indicator of a combined VSRAD and 1H MRS marker.

ROC analysis showed an AUC of 0.94, sensitivity 93.1%, specificity 82.2% at the cut off of 0.89 in controls versus AD, and the AUC of 0.88, sensitivity 85.7%, and specificity 83.2% at the cut off of 0.90, in controls versus MCI-C (Fig. 6).
Fig. 5. ANOVA of $^1$H MRS metabolite ratio in the PCG in the 118 subjects without apparent cognitive decline and apparent brain atrophy. The value of the NAA/MI ratio significantly decreased in MCI-NC, MCI-C, AD, and DLB compared with controls.

Fig. 6. Diagnostic value of [Z-score: severity of MTA/(NAA/MI)] as a referential indicator of a combined VSRAD and $^1$H MRS marker. The value of [Z-score: severity of MTA on VSRAD/(NAA/MI) on $^1$H MRS] is calculated as a referential indicator of a combined VSRAD and $^1$H MRS marker. ROC analysis showed an AUC of 0.94, sensitivity 93.1%, specificity 82.2% at the cut off value of 0.89 in controls versus AD, and an AUC of 0.88, sensitivity 85.7%, and specificity 83.2% at the cut off value of 0.90 in controls versus MCI-C.
Correlation between VSRAD and 1H MRS results and the MMSE

The results of this analysis are shown in Fig. 7. There were significant correlations between MMSE scores and the severity of MTA ($r = -0.3303$, $p < 0.0001^{***}$), NAA/Cr ($r = 0.39153$, $p < 0.0001^{***}$), and MAA/MI ($r = 0.1934$, $p = 0.0003^{***}$). These results indicate that NAA/Cr and MAA/MI on 1H MRS reflect cognitive function and could be useful markers for monitoring clinical states when combined with VSRAD.

Correlation between the results of the MRI analysis and CSF biomarkers

The correlations between the severity of MTA on VSRAD and CSF biomarkers were analyzed. The severity of MTA was correlated with CSF $A_\beta_{42}$ ($r = -0.23$, $p = 0.0082^{**}$), p-tau ($r = 0.18$, $p = 0.0334^{**}$), and $p$-tau/$A_\beta_{42}$ ($r = 0.26$, $p = 0.0022^{**}$), but not with p-tau or $p$-tau/$A_\beta_{42}$. NAA/Cr on 1H MRS in the PCG showed weak correlations between NAA/Cr and CSF $A_\beta_{42}$ ($r = 0.1693$, $p = 0.0499$), and no significant correlation with p-tau or p-tau/$A_\beta_{42}$ (Fig. 8).

DISCUSSION

Our results demonstrate that the Z-score showing the severity of the MTA using VSRAD is useful for distinguishing MCI (MCI-NC and MCI-C) and AD patients from healthy controls. However, there was no statistical difference between MCI-NC and MCI-C on VSRAD alone, whereas the level of CSF $A_\beta_{42}$/p-tau was significantly different between MCI-NC and MCI-C. Therefore, we suggest that the combination of the severity of MTA as shown via the VSRAD Z-score and the 1H MRS metabolite ratio (NAA/Cr and NAA/MI) may serve as a surrogate marker of AD and could be used as a screening tool for AD and MCI due to AD, as previously reported.

To date, numerous studies have shown that structural MRI and 1H MRS in the brain were useful not only for early diagnosis and for tracking disease conditions, but also for detecting prodromal AD [19–32, 40–45]. Kantarci et al. [19] performed a large-scale follow-up study using 1H MRS in the PCG and quantitative MRI in 1,156 cognitively normal older adults over 5 years. They showed that 214 of 1,156 subjects had progressed to MCI or dementia in 2.8 years.
Fig. 8. Correlation between CSF biomarkers and MTA on VSRAD and the metabolite ratio on 1H MRS in the PCG. a) MTA correlated with CSF Aβ42 \( (r = -0.23, p = 0.008^{**}) \), CSF p-tau \( (r = 0.18, p = 0.033^{*}) \), and CSF p-tau/CSF Aβ42 \( (r = 0.26, p = 0.002^{**}) \). b) NAA/Cr ratio correlated weakly with CSF Aβ42 \( (r = 0.17, p = 0.048^{*}) \), but was not correlated with CSF p-tau and CSF p-tau/CSF Aβ42. c) There was no correlation between CSF biomarkers and the NAA/MI ratio.

and demonstrated that decreased hippocampal volume and NAA/MI were independent predictors of MCI [19]. Our results showing that decreased hippocampal volume on VSRAD and decreased NAA/MI on 1H MRS in the PCG were risk factors for the development of AD may be overall compatible with previous
reports [19–32, 40–45]. Kantarci et al. [22] compared antemortem 1H MRS metabolite measurements and postmortem neuropathologic findings of AD including Braak neurofibrillary tangle stages and neuritic plaque scores. They demonstrated that decreased NAA/Cr and NAA/MI ratios and increased MI/Cr were correlated with the severity of AD pathology. Further, the authors concluded that the decreased NAA/MI ratio was associated with ongoing neurodegenerative processes in AD, indicating that 1H MRS measurements are potential noninvasive imaging markers for dementia due to AD [22].

Moreover, Kantarci et al. [51] also studied the relationship between metabolites on 1H MRS and Aβ burden using Pittsburgh compound B (PIB)-PET in 311 cognitively normal elderly persons. They revealed that increased MI/Cr and Cho/Cr ratio were associated with the preclinical pathologic process in the AD cascade [46].

As for structural MRI, VBM-MRI has been used for early diagnosis and for monitoring disease progression in AD [8–15]. Various techniques and software for VBM-MRI have been developed. SPM8 plus DARTEL (VSRAD) was developed by Matsuda et al. as VSRAD [9, 11]. VSRAD could capture the MTA known as hippocampal atrophy, which reflects the specific structural change seen in AD, and automatically evaluate the severity of MTA using a Z-score map. VSRAD is a popular technique especially in Japan because it is easy to handle, processing time is short, it is a costless and non-invasive technique for patients, and it is available in clinical practice. Therefore, VSRAD plus 1H MRS using regular 1.5-T MRI may be useful for screening subjects at risk for dementia due to AD. 1H MRS is superior in terms of detecting biochemical abnormalities preceding structural changes in the brain. 1H MRS in the PCG could show abnormal changes of each metabolite peak in MCI, AD, and the preclinical stage of AD compared with healthy controls. It has been considered that a decreased NAA/Cr ratio reflects decreased neuronal integrity, increased Cho/Cr ratio suggests increased membrane turnover due to AD, increased MI/Cr may reflect glial activation or neuroinflammation associated with neuronal degeneration, and decreased NAA/MI is a sensitive marker reflecting pathological changes and prognosis of MCI and AD [19–24, 31, 32].

The 1H MRS studies of familial AD with genetic mutations are convincing and may be applied to sporadic cases as well [31, 48]. Godbolt et al. [47] reported 1H MRS in the PCG in presymptomatic mutation carriers (PMCs) of presenilin 1 (PS1) or amyloid-β protein precursor (AβPP) mutations. They showed a significant decrease of NAA/Cr and NAA/MI in PMCs compared to controls, and concluded that metabolic changes are detectable in PMCs years before the expected onset of AD [47]. Kantarci et al. [48] studied subjects with microtubule-associated protein (MAPT) mutations. They showed that symptomatic MAPT mutation carriers were characterized by a decreased NAA/Cr ratio, NAA/MI ratio, hippocampal atrophy, and an increased MI/Cr ratio, whereas presymptomatic MAPT mutation carriers had increased MI/Cr and decreased NAA/MI with an unchanged NAA/Cr ratio and hippocampal volume compared to controls. These results indicated that the MI/Cr ratio, a possible index of glial activation, precedes the decrease of NAA/Cr, a neuronal integrity and hippocampal atrophy [48]. Previous reports showing 1H MRS abnormalities in the PCG of PMCs support the clinical utility of 1H MRS for screening prodromal AD.

The main objective of this study was to validate that conventional CSF biomarkers, VSRAD, and 1H MRS in the PCG at baseline could be applied for differentially diagnosing dementia and are sufficient for capturing subjects at risk for AD in clinical practice. Next, we studied VBM-MRI using VSRAD and 1H MRS in the PCG in comparison with the results of CSF biomarkers. The severity of MTA evaluated as a Z-score clearly distinguished AD, MCI-C, and MCI-NC patients from controls. However, VSRAD could not distinguish MCI-C from MCI-NC. Our results showed VSRAD could be a surrogate marker for screening MCI/AD patients. We showed that the ratio of NAA/MI at baseline could significantly distinguish AD, MCI-C, and DLB patients from controls and MCI-NC patients (Fig. 3a–c). ROC analysis showed that the ratio of NAA/Cr and NAA/MI could be good markers for the early diagnosis of MCI-C and AD (Fig. 3d), as previously reported. Our results showed a significant negative correlation between MMSE scores and the severity of MTA (Z-score) on VSRAD (r = −0.33, p < 0.0001***), NAA/Cr (r = 0.39, p < 0.0001***), and NAA/MI (r = 0.19, p < 0.0003***). These results suggested that VSRAD 1H MRS is useful for not only early screening of dementia due to AD, but also for tracking disease severity and monitoring the efficacy of drugs objectively.

Moreover, 118 of the 228 subjects (51.7%) were without apparent medial temporal atrophy (severity of MTA on VSRAD: Z-score ≤ 1.5 at baseline). At 3 years after baseline, these 118 subjects consisted of 79 controls, 7 MCI-NC, 11 MCI-C, 8 DLB, 1 NPH, and 6 OND patients. In these 118 subjects, studies using
VSRAD and $^1$H MRS at baseline revealed that only the NAA/MI ratio on $^1$H MRS could clearly differentiate MCI-C, AD, and DLB patients from controls. Especially in MCI, the NAA/MI ratio on $^1$H MRS could capture future MCI-C accurately compared to VSRAD alone. Thus, our results indicate the diagnostic utility of the NAA/MI ratio on $^1$H MRS. Kantarcı et al. [19] suggest that subjects with preserved hippocampal volumes, but low NAA/MI ratios, who progressed to MCI, may have atypical AD, a non-AD degenerative pathology, or cerebrovascular pathology. In such atypical cases, examinations of the CSF biomarkers, 18-Fluoro-deoxyglucose-PET, and PIB-PET studies will be required.

In this regard, we showed a significantly decreased NAA/Cr ratio and an increased MI/Cr ratio in the PCG without apparent MTA in subjects with DLB. These results are incompatible with previous reports showing no apparent decrease in the NAA/Cr ratio and an increased Cho/Cr ratio on $^1$H MRS in the PCG in DLB patients [31, 49]. The reason for these incompatible results in DLB patients is unclear; it is possible that the number of DLB subjects in our study was too small, our diagnosis of DLB was inappropriate, or our DLB subjects might have some pathological overlap with AD. Although our results contrast with those in previous reports, if our findings in DLB are accurate, DLB can be differentiated from AD by using VSRAD and $^1$H MRS, and is specifically characterized by decreased NAA/Cr, MRS, and is specifically characterized by decreased NAA/Cr and NAA/MI ratios and NAA/MI ratios without apparent MTA at an early stage. Thus, further studies are needed.

We studied the relationship among MMSE scores, CSF biomarkers, VSRAD/$^1$H MRS, and APOE genotype. Our results showed a significant correlation between MMSE scores and VSRAD/$^1$H MRS. We showed a negative correlation between the severity of MTA and CSF $A_\beta$$_{42}$, and a positive correlation between the severity of MTA and CSF p-tau and p-tau/$A_\beta$$_{42}$ on VSRAD (Fig. 8). Whitwell et al. [15] demonstrated a significant correlation between high burdens of neurofibrillary tangles composed of hyper-phosphorylated tau at autopsy and the severity of MTA by VBM-MRI. Our results showing a correlation between CSF biomarkers and the severity of MTA on VSRAD is compatible with the results by Whitwell et al. [15]. As for the correlation between CSF biomarkers and metabolites of $^1$H MRS in the PCG, we found that there was a positive correlation between the NAA/Cr ratio and the level of CSF $A_\beta$$_{42}$, but there was no significant correlation between the NAA/Cr, Cho/Cr, MI/Cr, and NAA/MI ratios and CSF biomarkers. We speculate that the elevation of the Cho/Cr and MI/Cr ratios on $^1$H MRS in AD could be caused by an independent pathomechanism that is different from the abnormal deposition of $A_\beta$ and tau.

Our study does have several limitations. First, we determined the PCG as a target VOI of $^1$H MRS by referencing previous reports. However, recent studies by Watanabe et al. [50, 51] compared metabolite (NAA, MI) concentrations in the hippocampus and PCG, and demonstrated that hippocampal NAA concentration on $^1$H MRS was superior to that on PCG for distinguishing amnestic MCI and AD patients from healthy controls [50, 51]. Since Watanabe et al. [50, 51] indicated that changes in the hippocampal metabolite concentrations are important in AD, we also tried to target the bilateral hippocampus for $^1$H MRS, but failed because of technical problems, e.g., the atrophied hippocampus contained amounts of CSF that would require relatively long processing times compared to the PCG, and poor compliance of patients with dementia. We need to address these technical problems before we can measure hippocampal metabolite concentrations.

Second, MMSE scores were the only neuropsychological examination used in this study. Compared to studies like the Wechsler Memory Scale-R (WMS-R), the MMSE is relatively weak at evaluating higher cognitive functions [52, 53], and is not sufficient to study the correlation between cognitive functions and the results of VSRAD/$^1$H MRS. Watanabe et al. [54] studied the correlation between the WMS-R results and metabolite concentrations in the bilateral hippocampus and PCG. They showed that NAA and MI concentrations in the left hippocampus were associated with episodic memory dysfunction in patients with amnestic MCI and AD, and found strong correlations between the left hippocampal MI concentration and delayed recall subsets. In contrast, NAA and MI concentrations in the PCG may not be as strongly related to episodic memory like the concentrations in the hippocampus [54].

Third, we evaluated the peak height ratios of NAA, Cho, and MI to Cr for $^1$H MRS because Cr levels are typically stable in AD, and the Cr peak is generally used as an internal standard to adjust for atrophy- and acquisition-related variability [19–32, 40–45]. However, it has recently been reported that the absolute metabolite concentration is more reliable compared to metabolite ratios on $^1$H MRS. Kreis et al. [55] reported that the quantification of metabolites was more reliable compared to the metabolite ratio because of variable
Cr levels. Alger [56] also noted that since a change in both the numerator and denominator contribute to the change in a ratio the quantification of metabolites might be preferable over the use of ratios, as it can potentially reveal more subtle differences and may be more reliable. Importantly, Watanabe et al. [50, 51] compared the diagnostic accuracy between the absolute metabolite concentration and metabolite ratios using $^1$H MRS to discriminate AD patients from healthy controls. They demonstrated that quantification of the absolute metabolite concentration was superior to that of metabolite ratios [50, 51]. Li and colleagues showed that metabolite ratio measurements suffer from higher variability than the measurements of absolute metabolite concentrations [57]. Tumati et al. [58] performed a meta-analysis on the diagnostic value of $^1$H MRS in MCI. They suggested that the use of absolute metabolite concentrations rather than metabolite ratios might improve the reliability of $^1$H MRS because of the variability of metabolites in the brain.

Taken together, our study suggests that VSRAD combined with $^1$H MRS are potentially useful methods for the early diagnosis of dementia due to AD in clinical practice. However, our results should be interpreted carefully and there are still some issues to resolve. Thus, further studies that consider the above shortcomings and conflicting factors are needed to confirm the utility of VSRAD combined with $^1$H MRS as routine screening methods for dementia.

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SUPPLEMENTARY MATERIAL

The supplementary table and figures are available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-132786.

REFERENCES


