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RESEARCH PAPER

¹H-MR spectroscopy metabolite levels correlate with executive function in vascular cognitive impairment

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ABSTRACT

Background White matter hyperintensities (WMHs) are associated with vascular cognitive impairment (VCI) but fail to correlate with neuropsychological measures. As proton MR spectroscopy (¹H-MRS) can identify ischaemic tissue, we hypothesised that MRS detectable brain metabolites would be superior to WMHs in predicting performance on neuropsychological tests.

Methods 60 patients with suspected VCI underwent clinical, neuropsychological, MRI and CSF studies. They were diagnosed as having subcortical ischaemic vascular disease (SIVD), multiple infarcts, mixed dementia and leukoaraiosis. We measured brain metabolites in a white matter region above the lateral ventricles with ¹H-MRS and WMH volume in this region and throughout the brain.

Results We found a significant correlation between both total creatine (Cr) and N-acetylaspartyl compounds (NAA) and standardised neuropsychological test scores. Cr levels in white matter correlated significantly with executive function ($p=0.001$), attention ($p=0.03$) and overall T score ($p=0.007$). When lesion volume was added as a covariate, NAA also showed a significant correlation with executive function ($p=0.003$) and overall T score ($p=0.015$). Furthermore, while metabolite levels also correlated with total white matter lesion volume, adjusting the Cr levels for lesion volume did not diminish the strength of the association between Cr levels and neuropsychological scores. The lowest metabolite levels and neuropsychological scores were found in the SIVD group. Finally, lesion volume alone did not correlate significantly with any neuropsychological test score.

Conclusion These results suggest that estimates of neurometabolite levels provide additional and useful information concerning cognitive function in VCI not obtainable by measurements of lesion load.

arteriopathy with subcortical infarcts and leucoencephalopathy.⁸ The lack of specificity of findings on routine clinical scans has motivated the search for other neuroimaging modalities that may provide more rigorous criteria for VCI subtypes and other dementias. Among the more promising of these modalities is proton MR spectroscopy (¹H-MRS), with several studies revealing reductions in the neuronal metabolite N-acetylaspartate (NAA) in patients with VCI.^{9–14} However, the majority of these studies used the ratio of NAA to the combined signal from creatine (Cr) and phosphocreatine to evaluate NAA,^{10–12 15 16} and the assumption that Cr is stable in brain disorders has been challenged by recent reports.^{17–19}

To avoid the ambiguity of the NAA/Cr ratio in studies on VCI, ‘absolute’ concentrations of metabolites have been estimated.^{14 20} The purpose of the present study was to examine the correlations between white matter (WM) metabolite concentrations estimated by ¹H-MRS imaging (¹H-MRSI) and the scores from a clinical battery of neuropsychiatric tests in VCI patients. The correlations were compared with those between WMH volume in the subjects and test scores as well as with regression models with both metabolite concentrations and lesion volumes as covariates. Based on our previous study,¹⁰ showing that WMH volume may include both pathological and non-pathological tissue while metabolite concentrations are altered only in pathological tissue, we hypothesised that neuropsychological test scores would demonstrate stronger correlations with ¹H-MRSI findings than with WMH volume.

SUBJECTS AND METHODS**Subjects**

Sixty subjects were recruited between 2006 and 2010 from the neurology clinics at the University of New Mexico Hospitals and the Albuquerque Veterans Hospital, and enrolled in the study after obtaining informed consent. All aspects of this study were performed in compliance with the regulations of the University of New Mexico Institutional Review Board and Human Research Review Committee, and the Albuquerque Veterans Hospital Research Committee. Patients were diagnosed with VCI based on clinical history and imaging studies. Patients found to have other causes of WM lesions, such as vasculitis and multiple sclerosis, were excluded from the study. All patients were participating in a multimodal study of VCI and the clinical characteristics of the cohort have been previously reported.²¹ Patients

INTRODUCTION

Vascular cognitive impairment (VCI) subsumes a wide range of cognitive deficits caused by ischaemic brain lesions.¹ White matter hyperintensities (WMHs) on fluid attenuated inversion recovery (FLAIR) and T₂ weighted MRI are commonly seen in VCI patients, and the size of the lesions is used to indicate the extent of ischaemia.^{2–4} However, WMHs are found in a number of neurological disorders and their contribution to VCI symptoms is controversial.^{5 6} Furthermore, considerable inter-rater variability in the scoring of radiological findings in VCI has been reported⁷ and, to date, correlations between lesion load and neuropsychological testing results have been shown only for processing speed in one study on cerebral autosomal dominant



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Table 1 Diagnostic categories of the study patients

Category	Definition
I. VCI	All patients with suspected cognitive deficits and evidence of CVA on MRI
II. Subgroups of VCI	
1. Multiple strokes	Stroke-like events with supportive evidence of one or more strokes on MRI. Lacunar infarcts in basal ganglia only
2. Subcortical ischaemic vascular disease	Extensive white matter lesions with or without lacunar infarcts in the basal ganglia
3. Hypoxic hypoperfusion	Large white matter lesions with evidence of severe hypotensive episode due to drugs or operative procedures
4. Mixed AD/VCI	Memory loss greater than executive dysfunction with symmetric white matter lesions
III. Leukoaraiosis	White matter lesions without evidence of VCI

AD, Alzheimer's disease; CVA, cerebrovascular accident; VCI, vascular cognitive impairment.

included were on average 61 (SD=15.94) years old and 27 (48%) of the patients were men. All patients underwent a neuropsychological screening test to determine their ability to understand the nature of the study. All subjects underwent similar assessments consisting of physical and neurological examinations by one of the study neurologists and basic laboratory testing to rule out other causes of dementia. A complete battery of neuropsychological testing was performed on all patients.

Most patients were followed for 1–2 years with repeat neurological and neuropsychological testing in order to improve diagnostic accuracy. The neurologists made consensus diagnoses. Diagnostic categories used are shown in table 1. Patients had WM lesions and cognitive complaints that were suggestive of VCI. Patients with one or more strokes associated with cognitive decline were diagnosed as multiple infarcts. Several patients had single strategic infarcts. Subcortical ischaemic vascular disease (SIVD) was diagnosed when patients had a constellation of symptoms that included the following features: (1)vascular risk factors included hypertension, diabetes mellitus and hyperlipidaemia; (2) clinical findings were hyperreflexia and gait abnormalities; (3) neuropsychological abnormalities included executive function worse than memory with language intact; (4) imaging features were large WM lesions on MRI and no cortical strokes; and (5) the CSF finding was increased Albumin Index.^{22–24} Mixed VCI and Alzheimer's disease patients (MX) had evidence of cerebrovascular disease and prominent memory

loss. The overlap between MX and SIVD made separation less precise: in general, the MX group were older, had smaller more periventricular WMHs and in one patient had autopsy confirmation. Three patients had WM lesions secondary to hypoxic hypoperfusion; two related to drug overdose and one from hypotension during surgery. WM lesions on FLAIR MRI were extensive in SIVD, stroke-like in multiple infarcts and symmetric in MX. A group of patients had WMHs on MRI but a consensus diagnosis of VCI was not reached and they were classified as leukoaraiosis (LA). Table 2 shows the clinical characteristics of patients in the different diagnostic categories.

Only subjects who had completed both the spectroscopy portion of the MRI as well as the neuropsychological testing were included in the analyses. Four subjects had incomplete neuropsychological assessments. Six subjects did not complete the spectroscopy or the data were unusable due to movement or data collection errors. A total of 52 subjects had complete spectroscopic and neuropsychological data.

Methods

Neuropsychological examinations

Standardised measures of cognitive functioning were given to all patients in the study. All tests were administered and scored according to standard procedures for that test and were administered by a trained psychologist. Standardised (T) scores were calculated for each test using published norms for each test. Where applicable, Heaton norms were used via the Halstead–Reitan battery normative software.²⁵

Averaged composite T scores were calculated for each of the four cognitive areas of interest (memory, executive functioning, attention and language) as well as an overall composite of cognitive functioning. Tests for each composite included: memory (Hopkins Verbal Learning Test-Delay, Rey Complex Figure Test-Long Delay), executive (Digit Span Backwards, Trail Making Test B, Wisconsin Card Sorting-Total errors), attention (Digit Span Forward and Trail Making Test A) and language (Boston Naming 60 item test, Controlled Oral Word Association). The overall composite included all of these composites averaged.

Anatomical MRI acquisition

The MRI investigation was performed with a 1.5 T Siemens Sonata scanner with a standard eight channel array head coil (Siemens AG, Erlangen, Germany). After the localiser images were acquired, T1 weighted, T2 weighted and FLAIR images were acquired using the following parameters: T1 weighted three-dimensional MP RAGE (magnetisation prepared with

Table 2 Findings in different diagnostic categories: subcortical ischaemic vascular disease, mixed vascular cognitive impairment/Alzheimer's disease, multiple infarcts and leukoaraiosis

Diagnosis	Hypertension (n (%))	Diabetes mellitus (n (%))	Gait (n (%))	Reflexes (n (%))	Executive function (mean (SD))	Memory (mean (SD))	MMSE (mean (SD))	NAA (mean (SD))	Lesion volume (mm ³) (mean (SD))	Albumin Index (n=41) (mean (SD))
SIVD (n=18)	11 (60)	6 (33)	13 (72)	14 (78)	42.22 (9.07)	41.11 (11.60)	27.61 (2.52)	10.70 (1.44)	42057 (26132)	7.80 (2.25)
MX (n=6)	5 (83)	2 (33)	4 (67)	3 (50)	41.17 (5.88)	38.00 (14.04)	24.83 (4.12)	10.60 (0.93)	36552 (14015)	6.08 (4.14)
MI (n=8)	6 (75)	1 (13)	6 (75)	7 (88)	40.00 (7.43)	39.00 (11.63)	28.75 (1.28)	12.30 (1.15)	12001 (12238)	7.53 (1.66)
LA (n=17)	5 (29)	2 (12)	8 (47)	9 (53)	44.82 (7.23)	45.29 (8.59)	27.53 (2.32)	12.44 (1.07)	16774 (16480)	4.67 (2.36)
Normal values	0	0	0	0	>45	>45	>25	>12	0	<6

LA, leukoaraiosis; MI, multiple infarcts; MX, mixed vascular cognitive impairment/Alzheimer's disease; MMSE, Mini-Mental State Examination; NAA, N-acetylaspartate; SIVD, subcortical ischaemic vascular disease; VCI, vascular cognitive impairment.

rapid acquisition gradient recalled echo): sagittal plane, TR/TE 12/4.76 ms, FOV 220 mm×220 mm, slice thickness 1.0 mm, slice gap 1.0 mm, number of slices 128, flip angle 20°, matrix 256×256, number of averages 1 and pixel bandwidth 110 Hz; T2 weighted two-dimensional turbo spin echo: axial plane, TR/TE 9040/64 ms, FOV 220 mm×220 mm, slice thickness 1.5 mm, echo train length 5, slice gap 1.0 mm, number of slices 120, matrix 192×192, number of averages 1 and pixel bandwidth 150 Hz; two-dimensional FLAIR: axial plane, TR/TE/IR 6000/358/2100 ms, FOV 220 mm×220 mm, slice thickness 1.5 mm, echo train length 107, number of slices 120, matrix 192×192, number of averages 2 and pixel bandwidth 745 Hz.

WMH measurement

WMH volume in whole brain was measured in FLAIR images using the software package JIM (V3.0, Xinapse Systems Ltd, Northants, UK, <http://www.xinapse.com>). Additionally, the fractional WMH volume just within the ¹H-MRSI region of interest (WMH volume/(WMH+grey matter+WM volume)) was assessed. Figure 1 shows representative FLAIR MRI images from each diagnostic group.

¹H-MRSI acquisition

¹H-MRSI was performed with a phase encoded version of a point resolved spectroscopy sequence (PRESS) with or without water presaturation (TR/TE=1500/135 ms, FOV=220×220 mm, slice thickness=15 mm, circular k space sampling (radius=24), total scan time=9 min 42 s). The nominal voxel size was 6.88×6.88×15 mm³ after zero filling in k space to 32×32 samples. Both water suppressed and water non-suppressed data sets were collected, allowing quantification of metabolites using the water non-suppressed signal as a concentration reference. The volume of interest (VOI) was established by the PRESS volume selection gradients and prescribed with a fast spin echo image to lie immediately above the lateral ventricles and parallel to the AC-PC line. To minimise the inclusion of voxels with chemical shift errors involving other resonances, the outermost rows and columns of the VOI were excluded from analysis. Adjustment of the magnetic field homogeneity within the VOI was performed with the Sonata three-dimensional shimming routine. Water suppression was achieved with chemical shift selective pulses.

¹H-MRSI data processing

After zero filling to 32×32 points in k space, applying a Hamming filter with a 50% window width and three-dimensional spatial Fourier transformation, the time domain ¹H-MRSI data were analysed using LCModel,^{19–26} using the unsuppressed water signal as a concentration reference. The results from LCModel were corrected for grey matter (GM),

WM, CSF and lesion content (partial volume effects), as previously reported.²⁷ Briefly, lesion maps were generated from FLAIR images by manual selection of hyperintense pixels within the ¹H-MRSI VOI using image processing software. GM, WM and CSF maps were generated by segmentation of the T1 weighted image with SPM5 (<http://www.fil.ion.ucl.ac.uk/spm>), using the WMH map as a mask to exclude the lesion pixels from the classification process. The WMH, GM, WM and CSF maps were then adjusted to the resolution of the ¹H-MRSI data by convolving the tissue maps with the theoretical ¹H-MRSI point spread function and the fractions of each tissue type and CSF in each ¹H-MRSI voxel were determined. These fractions, along with values for the water proton T1, T2 and density associated with each fraction (taken from published reports²⁷) were used to correct the water signal for partial volume effects and relaxation attenuation. As WMHs are generally isointense relative to GM in T1 and T2 weighted images, the water proton density and relaxation times of water protons in WMHs were assumed to be equivalent to those in GM. If the MR properties of water in WMH were, in fact, identical to normal appearing WM, this assumption would lead to an underestimation of the NAA concentration of less than 15% in a voxel containing only lesion. A similar error limit would apply to the estimate of Cr. Therefore, our assumptions with respect to the use of water as a concentration reference could not account for the correlation of metabolites with neuropsychological T scores observed in this work.

¹H-MRSI voxels with a predominance of WM (>66%) were classified as WM and those with a predominance of GM (>66%) were classified as GM. WM concentrations of total N-acetyl containing compounds (primarily NAA and N-acetylglutamylaspartate and here referred to simply as NAA), choline containing metabolites (Cho) and creatine+phosphocreatine (Cr) are reported. Figure 2 shows the location of regions of interest and a representative ¹H-MRS spectra.

Statistics

Bivariate Pearson correlation and multiple linear regression analyses involving all metabolites and WMH volume were performed with standard statistical software. Owing to the relatively high number of outliers in WMH volume across the sample, the square root of this measure, resulting in a distribution closer to normal, was used in analyses.

RESULTS

Exploratory Pearson correlation analyses, uncorrected for multiple comparisons, revealed several significant correlations among metabolite levels, the square root of WMH volume and neuropsychological scores (table 3). Scatterplots of NAA and Cr

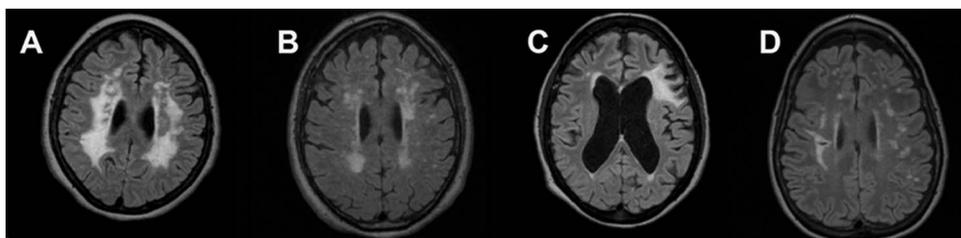
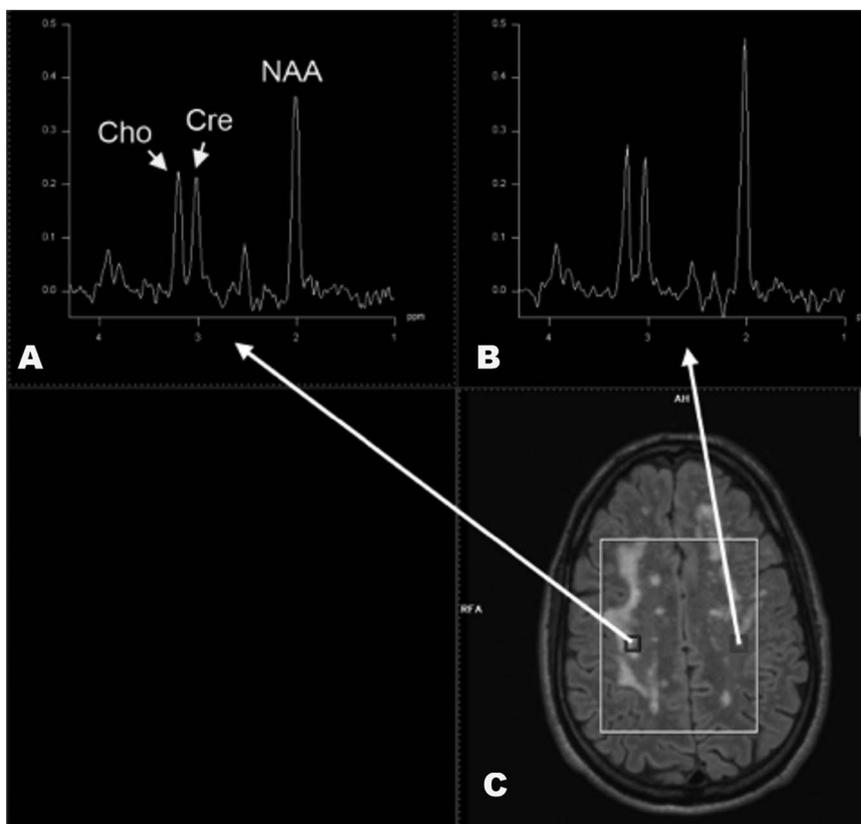


Figure 1 Representative fluid attenuated inversion recovery (FLAIR) MRI from the diagnostic groups. (A) Patient with subcortical ischaemic vascular disease. (B) Mixed vascular cognitive impairment/Alzheimer's disease. (C) Multiple infarcts primarily in the cortex or basal ganglia. (D) A representative leukoaraiosis patient with white matter changes and one small lacunar-like area, which was not sufficient to place the patient in the multiple stroke category.

Figure 2 ¹H-MR spectroscopy imaging spectra from a patient with subcortical ischaemic vascular disease. Multiple voxels are recorded in the white matter above the ventricle. (A) Characteristic peaks from an affected region. There is reduced N-acetylaspartate (NAA) but relatively normal choline (Cho) and creatine (Cr). (B) Spectra from a non-affected region of white matter. (C) Fluid attenuated inversion recovery (FLAIR) image showing the regions of white matter hyperintensities.



levels versus WMH volume are shown in figure 3. The square root of WMH volume correlated strongly with NAA and Cr (figure 3), and less strongly with Cho. Similar relationships were found between WMH volume (without transformation) and metabolites and with NAA and Cr and the square root of the fractional WMH volume within the ¹H-MRSI region of interest (results not shown). The latter result was expected as WMH fraction within the ¹H-MRSI region of interest was found to correlate significantly with whole brain WMH volume. Scatterplots of Cr and NAA versus neuropsychological testing and associated regression analyses also show that Cr levels correlate with multiple cognitive test scores (executive, attention and overall function T scores) and demonstrate a trend

($r=0.272$, $p=0.051$) with memory T score (figure 4B, C). Neither WMH volume nor its square root correlated significantly with any cognitive T score (figure 4A). No correlation was found between any cognitive score and the square root of

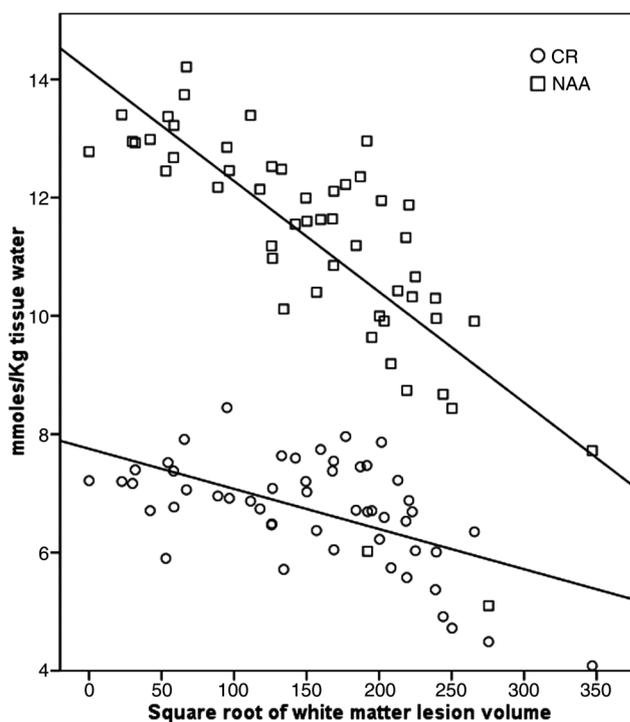


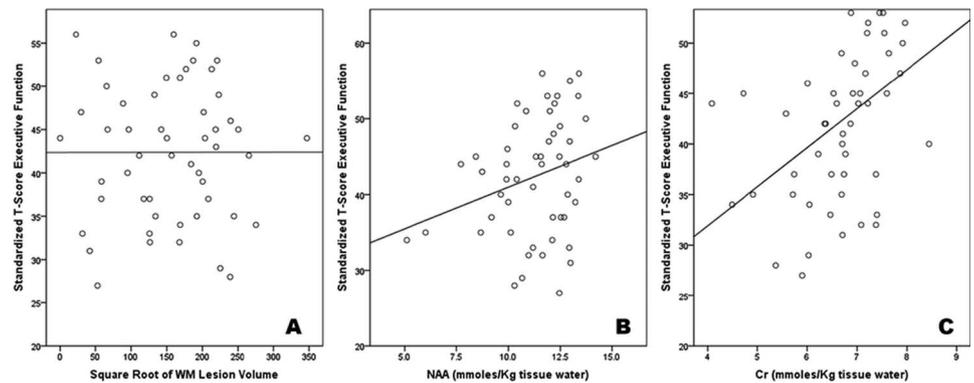
Figure 3 Scatterplots of N-acetylaspartate (NAA) and creatine (Cr) versus the square root of white matter hyperintensities volume. Significance is $p<0.001$ for both metabolites.

Table 3 Pearson correlations

		Lesion*	NAA	Cho	Cr
Executive T score	r	0.001	0.267	0.122	0.453†
	p	0.994	0.056	0.390	0.001
Memory T score	r	0.085	0.107	-0.017	0.272
	p	0.547	0.449	0.905	0.051
Attention T score	r	-0.074	0.184	0.193	0.302‡
	p	0.604	0.191	0.171	0.030
Language T score	r	-0.078	0.209	0.088	0.177
	p	0.583	0.137	0.536	0.208
Overall T score	r	-0.009	0.228	0.093	0.367†
	p	0.947	0.104	0.514	0.007
Lesion*	r	1.0	-0.756†	-0.386†	-0.567†
	p	—	<0.001	0.005	<0.001

*Square root of white matter hyperintensities volume.
 †Correlation is significant at the 0.01 level (two tailed).
 ‡Correlation is significant at the 0.05 level (two tailed).
 Cho, choline; Cr, creatine; NAA, N-acetylaspartate.

Figure 4 (A) Square root white matter (WM) hyperintensities volume versus executive function T score (results are not significant with $p=0.765$), (B) N-acetylaspartate (NAA) and (C) creatine (Cr) versus executive function T score. Significance is $p=0.056$ for NAA and $p<0.001$ for Cr.



the fractional WMH volume within the $^1\text{H-MRSI}$ region of interest. On the other hand, NAA demonstrated a correlation that approached significance ($p=0.056$) only with the executive T score. Cho was not related to any of the T scores. Plotting executive function against NAA and Cr for the LA and SIVD groups showed that Cr was significantly correlated in both groups, but that NAA was not significant for either (figure 5).

As lesion volume may be a confounding factor in the analysis of metabolites, we examined the metabolite effect while adjusting for the effect of WMH volume on cognitive scores. To accomplish this, the WMH volume square root was entered as a covariate with either WM NAA, Cr or Cho in linear regression models predicting the various T scores (executive, memory, attention, language or overall cognitive function). Table 4 shows the p values and squared regression coefficients (R^2) of the models with metabolite terms that were significant ($p<0.05$). With the WMH term included in the model, many of the metabolite effects are stronger than the bivariate correlations: Cr predicts executive, attention, memory and overall T scores and NAA significantly predicts executive and overall T scores, with trends for significant effects for memory and language T scores. The executive function T score is the cognitive test score best predicted by either NAA or Cr in these models, accounting for 17% and 30%, respectively, of the variance in the T score, while the regression coefficients for models with both Cho and

WMH volume are non-significant. The lesion term in the covariate models contributes significantly to the prediction of a T score when entered with Cr for predicting the executive T score or when entered with NAA for predicting the executive T score. Finally, as age has also been shown to influence neurometabolite levels,²⁸ the effect of patient age on the relationship between metabolite levels and cognitive scores was examined by entering age as a covariate with metabolite level. The effect of age was only significant in the regression model of predicting Cho by executive functioning ($R^2=0.121$, $p=0.04$).

DISCUSSION

In the present study, $^1\text{H-MRSI}$ estimates of WM levels of NAA and Cr in a region above the lateral ventricles were found to correlate significantly both with neuropsychological test T scores and WMH volume in a sample of 52 patients with VCI. In contrast, WMH volume alone failed to correlate significantly with any neuropsychological test T score. Furthermore, when the metabolite T score analysis was adjusted for WMH volume, lower p values for the associations between metabolite levels and T scores were generated. After adjusting for WMH volume, Cr correlated significantly with executive function, memory, attention and overall T scores while NAA correlated significantly with executive function and overall T scores in this sample. These results suggest, therefore, that estimates of neurometabolite levels provide additional and

Figure 5 N-acetylaspartate (NAA) and creatine (Cr) versus executive function for different diagnostic categories. (A) Cr versus executive function for leukoaraiosis (LA). (B) Cr versus executive function for subcortical ischaemic vascular disease (SIVD). (C) NAA versus executive function for LA. (D) NAA versus executive function for SIVD. Cr was significant for both LA and SIVD, while NAA was not significant for either. WM, white matter.

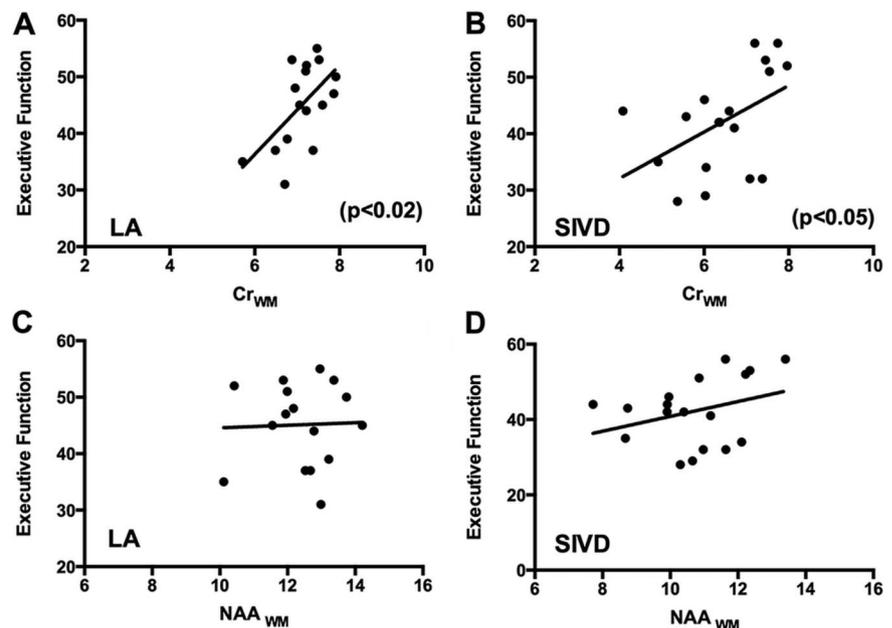


Table 4 Linear regression with metabolite and the square root of white matter hyperintensities volume

T score	Predictors	Metabolite		Lesion		Model	
		p Value	β	p Value	β	R ²	p Value
Executive	Cr+lesion	<0.001	0.668	0.012	0.380	0.303	<0.001
Attention	Cr+lesion	0.024	0.383	0.386	0.143	0.105	0.066
Memory	Cr+lesion	0.005	0.472	0.031	0.353	0.158	0.015
Language	Cr+lesion	0.255	0.196	0.845	0.033	0.032	0.448
Overall	Cr+lesion	0.001	0.532	0.067	0.292	0.192	0.005
Executive	NAA+lesion	0.003	0.625	0.021	0.474	0.167	0.011
Attention	NAA+lesion	0.165	0.301	0.165	0.154	0.044	0.331
Memory	NAA+lesion	0.061	0.402	0.070	0.389	0.076	0.143
Language	NAA+lesion	0.104	0.351	0.380	0.188	0.059	0.226
Overall	NAA+lesion	0.015	0.516	0.070	0.381	0.114	0.051

Cr, creatine; NAA, N-acetylaspartate.

useful information concerning cognitive function in VCI not obtainable by measurements of WMH volume.

In agreement with previous ¹H-MRS studies on brain metabolites in VCI, we observed that WM concentrations of NAA, Cr and Cho all correlated negatively with WMH volume.¹⁴ This finding is consistent with the expected ischaemic aetiology of these lesions, leading to metabolic dysfunction or cell death in these regions. However, in a previous report we showed that T2 weighted MRI WMHs in healthy elderly subjects had normal metabolite concentrations,¹⁰ indicating that the tissue was not metabolically compromised in the WMHs of these subjects. Furthermore, studies on different neuropathologies have shown altered metabolism in normal appearing WM.^{29–31} Hence although ischaemic lesions are expected to exhibit reduced NAA, Cr and Cho, the surrounding normal appearing tissue may also exhibit reduced metabolism, while non-ischaemic WMHs may exhibit normal metabolite levels. This suggests that the direct assessment of metabolism via ¹H-MRS may be a better index of brain function than WMH volume alone, as the metabolic status of all lesions need not be uniformly reduced nor all normal appearing tissue be metabolically 'normal'. This has been demonstrated, for example, to be the case in multiple sclerosis, in which NAA levels in patients have been shown to be a better predictor of symptoms than lesion load.³²

We found that significant correlations between neuropsychological tests scores and either NAA or Cr were mainly in the set of subtests of the neuropsychological examination that is generally abnormal in patients with VCI—namely, executive function. The finding that total Cr levels predict neuropsychological scores in this sample and, indeed, more consistently than NAA before adjusting for WMH volume, may be related to the central role of creatine and phosphocreatine in cellular energy metabolism, coupled with the underlying vascular–ischaemic causes of VCI, or simply due to increased measurement variance in NAA relative to that of Cr. Creatine and phosphocreatine compose the high energy phosphate buffering system in mammalian cells, which is critical for maintaining high levels of ATP. Hence, among many other energy roles, creatine and phosphocreatine are essential for maintaining ionic homeostasis in brain cells. As noted above, many previous reports have assumed that the total creatine signal is stable, even during metabolic depression. This is because, on increased energy demand during normal metabolism, phosphocreatine is converted to creatine (as ADP is converted into ATP), leaving the total creatine signal

constant. However, the conditions of normal metabolism may not be present in ischaemic tissue. Accordingly, lower Cr may simply reflect the extreme metabolic depression of ischaemic tissue. As Cr also gives rise to one of the strongest signals in the MRS spectrum, it may act as a particularly sensitive marker of this depression, on a par with or, in the case of the present clinical sample, even better than NAA. Regardless of the underlying reason for the superior sensitivity of Cr over NAA in predicting cognitive performance in the present study, a conclusion that can be reached is that, like NAA, Cr is reduced in ischaemic tissue, in agreement with past MRS studies that measured absolute measures of metabolites.

In a study comparing Alzheimer's disease patients, Binswanger's disease patients and healthy subjects with single voxel ¹H-MRS in several grey and WM regions, Watanabe *et al*²⁰ observed NAA and Cr differences between Binswanger's disease and healthy subjects across all regions, with NAA and Cr nearly 40% lower in the Binswanger's disease group. However, the authors did not compare their metabolic data with neuropsychological data. Nitkunan *et al*,¹⁴ on the other hand, observed significantly lower NAA in their sample of subcortical vascular disease subjects relative to healthy subjects, but no significant difference in Cr or correlations with neuropsychological measures in a short echo ¹H-MRSI study on a supraventricular region of brain. It is difficult to reconcile these differences, other than by ascribing them to sample differences (sample size, brain regions examined and types of patients). The Binswanger's disease sample of Watanabe *et al* may have included more patients with greater WM pathology than the subcortical vascular disease sample of Nitkunan *et al*, who noted that the absence of significant correlations between cognitive tests and metabolites in their study may have been related to their patient sample. Another possible difference between the present and previous studies is that we did not include a control group and used a longer pulse sequence echo time (135 ms) than studies of Watanabe *et al* or Nitkunan *et al*. A longer echo time sacrifices the ability to measure complex J couple signals, such as the myoinositol signal but, in fact, simplifies the spectrum and hence the measurement of NAA, Cho and Cr.

The SIVD group in our patient cohort most closely resembles the Binswanger's group in the study of Watanabe *et al*.²⁰ SIVD patients had the lowest values of NAA and Cr, the largest WM lesion volumes on FLAIR and abnormalities on neuropsychological testing, mainly in executive function. The diagnosis of SIVD remains controversial, and in reality is a continuum rather than a discrete category represented in our criteria shown in table 2. Although patients were categorised clinically into subgroups of VCI, NAA and Cr values were taken as a continuum of values across all diagnostic categories. Those with LA had the highest values for NAA, which were consistent with the control values obtained by us in other studies and allowed comparison with the SIVD group that had the lowest NAA values.³³ Finally, we note that other MRI modalities may characterise lesions better than the FLAIR images obtained in the current study. FLAIR only captures WMH shape and volumes while other MRI methods, such as diffusion tensor imaging, add other relevant information on tissue structure. Diffusion tensor imaging studies have shown a better correlation with cognitive measures than T2 lesion volumes and are highly correlated with NAA.³⁴

In summary, the results of this study demonstrate that measurement of WM NAA and Cr by ¹H-MRSI provides information that is more directly related to cognitive status in VCI patients than is WMH volume, as measured on FLAIR MRI, even though metabolite levels and WMH volumes are themselves strongly correlated. This finding agrees with our earlier

single voxel study on WMHs in VCI and age matched cognitively normal subjects,¹⁰ in which it was found that WMHs in cognitively normal individuals may not always demonstrate evidence of abnormal neurochemistry, and it is also consistent with studies on diverse other neuropathologies in which metabolic perturbation is observed in normal appearing tissue. Together these findings suggest that direct measurement of metabolic status in WM, both in normal and abnormal appearing tissue, is a more reliable determinant of the cognitive consequences of VCI pathology than is lesion load. Further studies are needed to explore and compare the relationships between symptoms and metabolism in subgroups of VCI and well matched healthy control groups, which may provide an evidence based categorisation of VCI.

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REFERENCES

- Moorhouse P, Rockwood K. Vascular cognitive impairment: current concepts and clinical developments. *Lancet Neurol* 2008;7:246–55.
- Schmidt R, Scheltens P, Erkinjuntti T, et al. White matter lesion progression: a surrogate endpoint for trials in cerebral small-vessel disease. *Neurology* 2004;63:139–44.
- Roman GC, Sachdev P, Royall DR, et al. Vascular cognitive disorder: a new diagnostic category updating vascular cognitive impairment and vascular dementia. *J Neurol Sci* 2004;226:81–7.
- Wiederkehr S, Simard M, Fortin C, et al. Validity of the clinical diagnostic criteria for vascular dementia: a critical review. Part II. *J Neuropsychiatry Clin Neurosci* 2008;20:162–77.
- Mungas D, Jagust WJ, Reed BR, et al. MRI predictors of cognition in subcortical ischemic vascular disease and Alzheimer's disease. *Neurology* 2001;57:2229–35.
- Jellinger KA. The enigma of vascular cognitive disorder and vascular dementia. *Acta Neuropathol* 2007;113:349–88.
- van Straaten EC, Scheltens P, Knol DL, et al. Operational definitions for the NINDS-AIREN criteria for vascular dementia: an interobserver study. *Stroke* 2003;34:1907–12.
- Duering M, Zieren N, Herve D, et al. Strategic role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL. *Brain* 2011;134(Pt 8):2366–75.
- Constans JM, Meyerhoff DJ, Norman D, et al. 1H and 31P magnetic resonance spectroscopic imaging of white matter signal hyperintensity areas in elderly subjects. *Neuroradiology* 1995;37:615–23.
- Brooks WM, Wesley MH, Kodituwakku PW, et al. 1H-MRS differentiates white matter hyperintensities in subcortical arteriosclerotic encephalopathy from those in normal elderly. *Stroke* 1997;28:1940–3.
- Waldman AD, Rai GS. The relationship between cognitive impairment and in vivo metabolite ratios in patients with clinical Alzheimer's disease and vascular dementia: a proton magnetic resonance spectroscopy study. *Neuroradiology* 2003;45:507–12.
- Martinez-Bisbal MC, Arana E, Marti-Bonmati L, et al. Cognitive impairment: classification by 1H magnetic resonance spectroscopy. *Eur J Neurol* 2004;11:187–93.
- Kantarci K, Petersen RC, Przybelski SA, et al. Hippocampal volumes, proton magnetic resonance spectroscopy metabolites, and cerebrovascular disease in mild cognitive impairment subtypes. *Arch Neurol* 2008;65:1621–8.
- Nitkunan A, Charlton RA, Barrick TR, et al. Reduced N-acetylaspartate is consistent with axonal dysfunction in cerebral small vessel disease. *NMR Biomed* 2009;22:285–91.
- MacKay S, Meyerhoff DJ, Constans JM, et al. Regional gray and white matter metabolite differences in subjects with AD, with subcortical vascular dementia, and elderly controls with 1H magnetic resonance spectroscopic imaging. *Arch Neurol* 1996;53:167–74.
- Kattapong VJ, Brooks WM, Wesley MH, et al. Proton magnetic resonance spectroscopy of vascular- and Alzheimer-type dementia. *Arch Neurol* 1996;53:678–80.
- Inglese M, Li BS, Rusinek H, et al. Diffusely elevated cerebral choline and creatine in relapsing-remitting multiple sclerosis. *Magn Reson Med* 2003;50:190–5.
- Hattingen E, Raab P, Franz K, et al. Prognostic value of choline and creatine in WHO grade II gliomas. *Neuroradiology* 2008;50:759–67.
- Gasparovic C, Yeo R, Mannell M, et al. Neurometabolite concentrations in gray and white matter in mild traumatic brain injury: an 1H-magnetic resonance spectroscopy study. *J Neurotrauma* 2009;26:1635–43.
- Watanabe T, Shiino A, Akiguchi I. Absolute quantification in proton magnetic resonance spectroscopy is superior to relative ratio to discriminate Alzheimer's disease from Binswanger's disease. *Dement Geriatr Cogn Disord* 2008;26:89–100.
- Taheri S, Gasparovic C, Huisa BN, et al. Blood-brain barrier permeability abnormalities in vascular cognitive impairment. *Stroke* 2011;42:2158–63.
- Ciulli L, Poggiosi A, Salvadori E, et al. The VAS-COG clinic: an out-patient service for patients with cognitive and behavioral consequences of cerebrovascular diseases. *Neurol Sci* 2012;33:1277–83.
- Caplan LR. Binswanger's disease—revisited. *Neurology* 1995;45:626–33.
- Bennett DA, Wilson RS, Gilley DW, et al. Clinical diagnosis of Binswanger's disease (see comments). *J Neurol Neurosurg Psychiatry* 1990;53:961–5.
- Heaton RK, Miller SW, Taylor MJ, et al. Revised comprehensive norms for an expanded Halstead-Reitan battery: demographically adjusted neuropsychological norms for African American and Caucasian Adults (HRB). *Psychological Assessment Resources, Inc.* v. 4.01.022.
- Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* 1993;30:672–9.
- Gasparovic C, Song T, Devier D, et al. Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magn Reson Med* 2006;55:1219–26.
- Pfefferbaum A, Adalsteinsson E, Spielman D, et al. In vivo spectroscopic quantification of the N-acetyl moiety, creatine, and choline from large volumes of brain gray and white matter: effects of normal aging. *Magn Reson Med* 1999;41:276–84.
- Moffett JR, Ross B, Arun P, et al. N-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. *Prog Neurobiol* 2007;81:89–131.
- Aboul-Enein F, Krssak M, Hoftberger R, et al. Reduced NAA-levels in the NAWM of patients with MS is a feature of progression. A study with quantitative magnetic resonance spectroscopy at 3 Tesla. *PLoS One* 2010;5:e11625.
- Filippi M, Rocca MA. Multiple sclerosis and allied white matter diseases. *Neurol Sci* 2008;29(Suppl 3):319–22.
- Bellmann-Strobl J, Stiepani H, Wuerfel J, et al. MR spectroscopy (MRS) and magnetisation transfer imaging (MTI), lesion load and clinical scores in early relapsing remitting multiple sclerosis: a combined cross-sectional and longitudinal study. *Eur Radiol* 2009;19:2066–74.
- Gasparovic C, Bedrick EJ, Mayer AR, et al. Test-retest reliability and reproducibility of short-echo-time spectroscopic imaging of human brain at 3T. *Magn Reson Med* 2011;66:324–32.
- O'Sullivan M, Morris RG, Huckstep B, et al. Diffusion tensor MRI correlates with executive dysfunction in patients with ischaemic leukoaraiosis. *J Neurol Neurosurg Psychiatry* 2004;75:441–7.