Associations Between Diffusion and Perfusion Parameters, N-Acetyl Aspartate, and Lactate in Acute Ischemic Stroke

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Background and Purpose—In acute ischemic stroke, the amount of neuronal damage in hyperintense areas on MR diffusion imaging (DWI) is unclear. We used spectroscopic imaging to measure N-acetyl aspartate (NAA, a marker of normal neurons) and lactate (a marker of ischemia) to compare with diffusion and perfusion values in the diffusion lesion in acute ischemic stroke.

Methods—We recruited patients with acute ischemic stroke prospectively and performed MR diffusion weighted (DWI), perfusion, and spectroscopic imaging. We coregistered the images, outlined the visible diffusion lesion, and extracted metabolite, perfusion, and apparent diffusion coefficient (ADC) values from the diffusion lesion.

Results—42 patients were imaged, from 1.5 to 24 hours after stroke. In the DWI lesion, although NAA was reduced, there was no correlation between NAA and ADC or perfusion values. However, raised lactate correlated with reduced ADC (Spearman ρ = 0.32, P = 0.04) and prolonged mean transit time (MTT, ρ = 0.31, P = 0.04). Increasing DWI lesion size was associated with lower NAA and higher lactate (ρ = −0.44, P = 0.003; ρ = 0.49, P = 0.001 respectively); NAA fell with increasing times to imaging (ρ = −0.3, P = 0.03), but lactate did not change.

Conclusion—Although larger confirmatory studies are needed, the correlation of ADC and MTT with lactate but not NAA suggests that ADC and MTT are better markers of the presence of ischemia than of cumulative neuronal loss. Further studies should define more precisely the rate of neuronal loss and relationship to diffusion and perfusion parameters with respect to the depth and duration of ischemia. (Stroke. 2009;40:767-772.)

Key Words: stroke ■ spectroscopy ■ metabolites ■ N-acetyl aspartate ■ lactate ■ ADC ■ CSI ■ DWI ■ PWI

Magnetic resonance (MR) diffusion and perfusion weighted imaging (DWI and PWI) could identify “at risk” tissue in patients with acute ischemic stroke. However, some initially abnormal areas on DWI may recover,1 and no risk” tissue in patients with acute ischemic stroke. However, some initially abnormal areas on DWI may recover,1 and no risk” tissue in patients with acute ischemic stroke. However, some initially abnormal areas on DWI may recover,1 and no risk” tissue in patients with acute ischemic stroke. However, some initially abnormal areas on DWI may recover,1 and no risk” tissue in patients with acute ischemic stroke. However, some initially abnormal areas on DWI may recover,1 and no risk” tissue in patients with acute ischemic stroke.
ities. Those that examined NAA and DWI found a wide range of NAA values within a narrow range of apparent diffusion coefficient (ADC) values and no clear correlation between NAA and ADC, but this analysis was only based on 20 patients.\textsuperscript{11,15} No studies examined the relationship between metabolites and both DWI and PWI parameters across the whole DWI-visible lesion.

As ADC and PWI values change immediately on the onset of ischemia, whereas neuronal death occurs after a period of ischemia, one explanation for the variability in reported ADC and PWI thresholds of viability, and in the range of NAA values for a given ADC value in previous studies,\textsuperscript{11,15} could be that DWI and PWI values mainly indicate the presence of ischemia but are only indirect markers of neuronal loss. Therefore, we used spectroscopy to determine in what way the changes in ADC and PWI values in the visible diffusion lesion indicate the amount of neuronal death.

**Methods**

**Patient Recruitment**

We prospectively recruited patients with acute cortical ischemic stroke admitted to our hospital stroke service without contraindications to MR imaging (MRI). An experienced stroke physician obtained a detailed history and neurological examination and measured the stroke severity according to the National Institutes of Health Stroke Scale (NIHSS, http://www.strokecenter.org/trials/scales/). Scanner availability limited recruitment to patients who could be scanned within normal working hours. We excluded patients who were too unwell to cooperate with MRI. The whole scanning protocol took 35 minutes, of which spectroscopy was 10 minutes. In patients who awoke with stroke or in whom the stroke was not witnessed, we either used the time of going to sleep or estimated the time of onset as the most likely time using all available evidence from carers. All patients were followed up at 3 months to determine functional outcome using the modified Rankin scale, blind to imaging findings. The study was approved by the Local Research Ethics Committee, and all subjects or their relatives gave written informed consent.

**MR Imaging and MRS**

Patients underwent MRI as soon as possible after admission but within 24 hours of stroke onset at the latest. We used a GE 1.5 T Signa LX (General Electric) scanner. We performed axial T2-weighted fast spin echo (T2W) and gradient echo imaging,\textsuperscript{17} axial T2-weighted spin-echo echo-planar imaging (EPI), axial dynamic susceptibility contrast PWI using gradient-echo EPI, and single slice point resolved spectroscopy (PRESS) proton MRS chemical shift imaging (CSI). For DWI, diffusion sensitizing gradients with scalar b values of 1000 s/mm\textsuperscript{2} were applied in 6 noncollinear directions with field of view (FOV) 240\times20 mm, 15 axial slices of thickness 5 mm, slice gap 1 mm, acquisition matrix 128\times128, echo time (TE) 97.4 ms, and repetition time (TR) 10 s. PWI was performed with a bolus injection of gadolinium (10 mL of 1 mmol/mL Gadovist or 20 mL of 0.5 mmol/mL Omniscan) using a power injector. The data were acquired over 85 s, collecting 34 volumes of 15 axial slices using the same slice parameters as DWI but with a TE of 30 ms and TR of 2.5 s. For spectroscopy, we used a single slice point resolved spectroscopy sequence. We centered the spectroscopy acquisition volume to cover the acute ischemic lesion as seen on the DWI, including as much contralateral normal cortex and white matter as possible and avoiding contamination from dura, skull, or subcutaneous fat. We used automatic shimming and water suppression, with FOV 320 mm, acquisition matrix 24\times24, TE 145 ms, TR 1000 ms, slices 1 cm thick, and about 10\times10 cm in the axial plane adjusted to fit the individual head size. For each phase encoding, 512 complex data points were acquired with a sampling interval of 1 ms.

**DWI and PWI Processing**

We converted the DICOM images to Analyze format (Mayo Foundation) on a Sun Ultra Sparc Station 10 (SUN Microsystems). We performed subsequent processing in MATLAB (The MathWork). We aligned and co-registered DWI, PWI, and CSI data to each other (using FLIRT, www.fmrib.ox.ac.uk/fs/1 or removed bulk patient motion and eddy current induced artifacts. We calculated DWI and ADC maps. Perfusion parameters were calculated from a gamma variate function fitted to the concentration time curves and corrected for arterial input function using both internal carotid arteries.\textsuperscript{18,19} We calculated cerebral blood flow (CBF), cerebral blood volume (CBV), and mean transit time (MTT) from the first moment of the curve (MTT), area under curve (CBV), and CBF (CBV/MTT) as the methods least prone to mathematical inaccuracies.\textsuperscript{19}

**MRS Processing**

We interpolated spectroscopic data to 10 mm\textsuperscript{3} voxels (32\times32 matrix), performed zero order phase correction using the residual water signal (bringing water to a chemical shift of 4.70 ppm), and removed residual water signal using the Hancel Lanczos singular value decomposition method.\textsuperscript{20} We Fourier-transformed the data and modeled the resulting spectra to extract NAA, choline, creatine, and lactate in absolute values (institutional units) using AMARES\textsuperscript{21} in MRUI.\textsuperscript{22} We excluded poor quality spectra if fitted line widths were <1 Hz or >10 Hz, if any metabolite peaks were more than 0.1 ppm offset from their expected values, if the voxels lay on the edges of the spectroscopy acquisition region, fell outside brain tissue, or contained CSF. We corrected metabolite peak areas for scanner drift and calibrated the institutional units using monthly quality assurance data.

**Regions of Interest (ROI)**

We outlined the acute ischemic lesion on DWI, on images with standard contrast settings, using Analyze software, blinded to other imaging and clinical data. Images were outlined by an experienced trained rater. We used the DWI image (not the ADC map) because we wished to determine metabolite parameters in the DWI-visible lesion, rather than within a single possibly arbitrary ADC threshold. A mirror image ROI was placed in the contralateral hemisphere. We superimposed the ROIs on the ADC, CBF, MTT, and CSI maps and extracted corresponding lesion and contralateral normal DWI, PWI, values, and metabolites (Figure 1). The DWI ROI covered the whole of the abnormality, whereas CSI was from the 10-mm slab. We were careful not to include CSI voxels lying at the edge of but mostly outside the ROI, in the ROI thereby minimizing partial volume effects. Relative perfusion values were obtained by dividing the stroke ROI values by those from the contralateral mirror image region.

**Statistical Analysis**

We used SPSS 11.0 for Windows (SPSS Inc) and nonparametric analyses (Wilcoxon Signed Rank Sum Test or Spearman Rank Correlation Coefficient, \(p\) with significance set at 0.05 (2 tailed), as the imaging data were not normally distributed (Kolmogorov-Smirnov test, \(P>0.01\)). We tested for independent associations between metabolites and diffusion/perfusion parameters using linear regression. We used absolute lesion metabolite and ADC values in the primary analyses, but repeated the comparison using the lesion/contralateral region metabolite ratios and [lesion NAA]/[lesion NAA+choline+creatine] for comparison with previous studies. We also examined subgroups imaged between 0 to 6, 6 to 12, and 12 to 24 hours.

**Results**

**Patients**

Of 65 potentially eligible patients, 15 were excluded (12 with hemorrhagic stroke; three with ischemic stroke were unable
to complete the imaging). Of the 50 patients with ischemic stroke and complete imaging, 5 had no visible DWI lesion and 3 did not have sufficiently good quality spectra (because of movement) for analysis. Therefore, 42 patients were included in the present analyses, with median age of 76 years (range 37 to 95 years, SD 11). Median time to imaging was 7.9 hours after stroke (range 1.5 to 24 hours), with 14/42 (33%) scanned within 6 hours, 13/42 (31%) between 6 and 12 hours, and the rest (15/42) scanned between 12 and 24 hours. Eleven patients awoke with stroke. The median NIHSS was 9 (range 1 to 29, SD 7.8).

**Metabolites in the DWI Lesion**

NAA and choline levels were significantly lower in the diffusion lesion than in contralateral normal brain (ipsilateral versus contralateral: NAA 96.0 versus 122.4, \( P = 0.0001 \); choline 63.6 versus 70.1, \( P = 0.015 \) Wilcoxon Rank Sum; Figure 2). Lactate was detected in all but one diffusion lesion (lactate ipsi versus contralateral: 42.5 versus 12.5, \( P = 0.0001 \)). Some lactate was detected in 10 patients in the contralateral region; in all cases this was less than in the diffusion lesion, all had large diffusion lesions and generalized cerebral atrophy. Creatine did not differ significantly between the lesion and contralateral normal brain.

**Metabolites, DWI and PWI Parameters**

There was no correlation between lesion NAA and ADC, MTT or CBF (Table 1). Higher lesion lactate was associated with lower lesion ADC (Spearman \( \rho = -0.32, P = 0.039 \)) and prolonged MTT (Spearman \( \rho = 0.31, P = 0.04 \)). There were no associations between choline or creatine and DWI or PWI values. These results did not change when we repeated these analyses using metabolite and ADC ratios (ie, relative values) instead of absolute values. Restricting the analyses to just those patients imaged within 6 hours still did not show any correlation between NAA and ADC, MTT, and CBF. Lactate correlated strongly with MTT (Spearman \( \rho = 0.74, P = 0.003 \)) and CBF (Spearman \( \rho = -0.78, P = 0.001 \)) but not ADC (Spearman \( \rho = -0.495, P = 0.07 \)). There was an association between \([\text{lesion NAA}] / [\text{lesion NAA} + \text{choline} + \text{creatine}]\) and reduced ADC (Spearman \( \rho = 0.434, P = 0.005 \)), but this composite ratio is difficult to interpret given that neither NAA nor choline are constant in acute stroke.

**Metabolites, Stroke Severity, Lesion Volume, Time From Stroke, and 3-Month Functional Outcome**

There was no association between any of the metabolites and the NIHSS score. Larger DWI lesion volume was associated with reduced NAA (Spearman \( \rho = -0.42, P = 0.006 \)) and
increased lactate (Spearman $r = 0.49$, $P = 0.001$) but not with choline or creatine. Longer times to scanning were associated with lower NAA (Spearman $r = -0.33$, $P = 0.03$) and larger lesion volumes (Spearman $r = 0.35$, $P = 0.023$) but not with lactate, choline, creatine, or ADC. There was no correlation between the metabolites, ADC, MTT, or CBF and 3-month Rankin score. However, the clinical parameter of NIHSS (measured at baseline) was associated with the 3-month Rankin score (Spearman $r = 0.665$, $P = 0.0001$), in keeping with the known strong association between stroke severity and functional outcome.

### Linear Regression Analysis

We tested for independent associations between NAA, lactate and diffusion/perfusion parameters, time to scan and stroke severity using linear regression (Table 2). MTT ($P = 0.02$) and DWI lesion volume ($P = 0.005$) were significant predictors in the lactate model and time from onset to scan ($P = 0.034$) in the NAA model.

### Discussion

We found no direct association between NAA as an index of neuronal loss and ADC, CBF, or MTT in the acute diffusion lesion, on either absolute or relative measures. However we did find associations between elevated lactate, reduced ADC, and prolonged MTT, which all indicate the presence of ischemia. The results did not change when the analysis was restricted to patients imaged within 6 hours of stroke. These results confirm that observations made in previous small MRS studies were correct: there was no correlation between NAA and ADC because the range of NAA values for a given ADC value was very wide. Therefore ADC and perfusion values are useful markers of the presence of ischemia. The lack of correlation with neuronal damage would explain the absence of agreement between previous studies on reported thresholds for salvageable/nonsalvageable tissue. The heterogeneity of ADC and PWI values within and around the acute stroke lesion, together with recovery of DWI-abnormal tissue in some cases, means that it is unlikely that any single DWI or PWI parameters will reliably indicate the amount of permanent tissue damage.

The study has limitations. Although large compared with all previous MRS studies and indeed with many MRI studies in stroke, it is relatively small compared with the variability of stroke. Despite efforts to recruit patients nonselectively, restrictions on MR availability to normal working hours meant that patients were only recruited if they could be scanned during working hours within the time window. However, we have no reason to think that patients admitted overnight were different to those seen during the day. As in many MR studies, not all patients were able to complete the scanning protocol. ROI analyses may be subject to partial volume effects, contamination by CSF or adjacent badly fitted spectra, even though patients with poorly fitted spectra were excluded from the outset, we were careful to avoid CSF contamination and careful to assign ROI voxels on the edge of the ROI so as to avoid partial volume averaging with normal tissue as much as possible. We used a single thick spectroscopic imaging slab, but centered the slab on the DWI slice that showed the acute stroke lesion at its most extensive, the spectroscopy slice covered the entire thickness of the DWI and PWI slice that showed the maximum lesion extent, plus 2.5 mm on either side, thereby capturing representative spectroscopic information across a large proportion of the stroke lesions. We have no reason to believe that lesion DWI or PWI or MRS values on slices outside the ROI were different from those sampled within the ROI, and the MRS slab provided much more information than the single voxel MRS used in previous studies. Acquisition of multiple adjacent spectroscopy slices would have taken too much time to be feasible in acute stroke. Although stroke lesions are known to be heterogeneous, ROI analyses are widely used because they provide a summary estimate of the average change in a parameter in the lesion, and in some circumstances have shown better correlation with final infarct size and infarct growth than pixel-based analyses. The correlations of metabolites with time after stroke should be interpreted cautiously, because these data come from different patients imaged at different times after stroke, not the same patients imaged serially. We may have estimated the time of onset as earlier than it actually was in those who awoke from sleep with stroke, as there is some evidence that the stroke most often occurs shortly before awaking rather than just after falling asleep, but we wished to be conservative. The multivariate analysis may adjust for some confounding factors, but not all and multiple comparisons should be cautiously interpreted. Thus, associations between time and ADC,
NAA, or PWI values may still be confounded by stroke severity or other factors, and these data should be regarded as exploratory. Larger studies with more patients imaged sequentially early after stroke would be required to be certain of overcoming these confounders reliably, although would be very difficult to do and might suffer from other sources of bias.

How does the present study compare with earlier work? Previous studies, mostly using single voxel techniques, compared metabolites with diffusion parameters but did not examine the association between metabolites and both diffusion/perfusion parameters. The single voxel approach samples less of the acute stroke lesion and allows a poorer comparison with DWI and PWI metrics than does the CSI technique used here. We confirmed the results of one previous study which showed a correlation between ADC and the ratio of [lesion NAA]: [NAA + choline + creatine] found in 6 patients, but metabolite ratios should be used with caution because lesion choline is not normal, and methods for quantifying metabolites are now well established. Others found reduced NAA in areas of cerebral blood flow reduction using single photon computed tomography or transcranial Doppler ultrasound but did not correlate metabolite and blood flow levels. The observation that larger diffusion lesions were associated with lower NAA and higher lactate values is consistent with previous studies showing that large lesions on T2 weighted imaging had lower NAA and higher lactate than small lesions.

We found very small amounts of lactate in the contralateral brain in some patients with large ischemic lesions and significant cerebral atrophy. This may represent leakage of lactate from large stroke lesions via CSF. As lactate has been found in normal brains of older subjects, the lactate in contralateral brain could simply be related to aging.

What do the present findings mean? NAA is found exclusively in functioning neurons. Several ischemia models (in mice, rats, monkeys) demonstrated an initial rapid decrease of NAA after induction of ischemia, then a further slower decline, and correlated closely with histological evidence of neuronal death. The NAA had fallen to 50% of normal by 6 hours and to 20% of normal by 24 hours, corresponding with similar proportions of nonviable neurons identified histologically. Thus NAA is very specific for neuronal loss in acute ischemia. This is in contrast to subacute times where there is some uncertainty about whether any residual NAA detected spectroscopically represents a few still viable neurons, neuronal debris, infiltrating microglia, or possibly some still viable neurons. This pattern of early NAA loss has been confirmed in patients.

In contrast, in animal models, the ADC value falls as neuronal and glial swelling develops, and then either remains low in persistent occlusion models until dead cells of all types are lysed, or rises in transient ischemia models due primarily to resolution of glial swelling. Indeed histological comparisons suggest that the ADC is a better marker of glial cell status than of neuronal viability, with the DWI signal (“whiteness”) being a better marker than the ADC of neuronal death, which is why we placed the ROI around the DWI-visible lesion rather than using an arbitrary ADC threshold. The lack of specificity of ADC values for neuronal death is also suggested by the wide range of values found in definite infarcts. PWI, DWI, and lactate are definite markers of early ischemia, but the lack of correlation with NAA suggest that they are not specific indicators of permanent tissue damage. The lack of correlation between ADC and neuronal loss as indicated by NAA may also be because the ADC falls very rapidly after symptom onset before rising, at different rates in different patients, to supra normal values. From any one “snapshot” in time, it is not possible to say whether the ADC is falling, at the nadir, or rising. Tissue with very low ADC values may recover in patients and animal models providing further evidence that no single ADC threshold is likely to discriminate salvageable from unsalvageable tissue. PWI values are also very heterogeneous in stroke lesions with no clear threshold for salvageable tissue. Therefore it would be difficult for a single “snapshot” of ADC or PWI values to indicate, very directly, neuronal loss or tissue salvageability.

MRS is still not suitable for routine assessment of acute stroke patients though is useful in research. From a clinical perspective, only NIHSS (not metabolites or PWI/DWI parameters) predicted 3-month outcome. This exploratory work suggests that more information is needed to determine whether, and how, imaging metrics of tissue damage can be used to assess tissue salvageability and guide patient management before treatment decisions can be based reliably on imaging indicators of tissue viability.

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Disclosures
None.

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