Measurement of Initial N-Acetyl Aspartate Concentration by Magnetic Resonance Spectroscopy and Initial Infarct Volume by MRI Predicts Outcome in Patients With Middle Cerebral Artery Territory Infarction

Anthony C. Pereira, MB, BChir; Dawn E. Saunders, MD; Victoria L. Doyle, PhD; J. Martin Bland, PhD; Franklyn A. Howe, DPhil; John R. Griffiths, DPhil; Martin M. Brown, MD

Background and Purpose—$^1$H MR spectroscopy can be used to study biochemical changes occurring in the brain in stroke. We used it to examine the relationship between metabolite concentration (N-acetyl aspartate [NAA], lactate, cholines and creatines), size of infarct, clinical deficit, and 3-month clinical outcome in patients with middle cerebral artery (MCA) territory infarction.

Methods—Thirty-one patients with acute MCA territory infarction were recruited within 72 hours of the onset of symptoms. Single-voxel short echo time stimulated echo acquisition mode spectroscopy was used to obtain metabolite data from the infarct core. Metabolite concentrations were determined with use of variable projection time domain-fitting analysis. Infarct size was determined with T2-weighted images. Patient outcome groups at 3 months were “independent,” “dependent,” or “dead.”

Results—All patients (100%; 95% CI 75% to 100%) who had an infarct $\geq$70 mL did poorly. Eighteen of 20 patients (90%; 95% CI 68% to 99%) with a core NAA concentration $<7$ mmol/L did poorly at 3 months, whereas 7 of 11 patients (64%; 95% CI 31% to 89%) with an initial NAA concentration $>7$ mmol/L did well. Combining these results showed that all patients who had an initial infarct volume $\geq$70 mL did poorly, irrespective of the NAA concentration. Of those patients with infarcts $<70$ mL, those who had a core NAA concentration $>7$ mmol/L did well (88%; 95% CI 47% to 100%), whereas those with a lower NAA concentration did poorly (80%; 95% CI 44% to 97%). There was no association between other metabolite concentrations and outcome.

Conclusions—Infarct volume and NAA concentration can together predict clinical outcome in MCA infarction in humans. (Stroke. 1999;30:1577-1582.)

Key Words: human ▪ outcome ▪ spectroscopy, nuclear magnetic resonance ▪ stroke

Stroke is the third most common cause of death and the most common cause of adult disability in Western countries. Recent advances in the management of acute stroke include thrombolysis and the use of organized stroke units. The prognosis of stroke is extremely variable, and it is difficult to predict the clinical outcome of the patient at the time of presentation. Better prediction of outcome would allow treatment to be targeted at those most likely to benefit. MRI allows precise localization of the region of infarction and is very sensitive to the early changes of cerebral ischemia. However, MRI provides mainly anatomic information about the size and site of the lesion and gives no information about the biochemical changes and severity of ischemia occurring within the region of imaging abnormality. Another MR technique, in vivo $^1$H MR spectroscopy ($^1$H MRS), allows the noninvasive study of the biochemical changes that accompany cerebral infarction and has the potential to allow measurement of the severity of ischemic damage.

The 2 metabolites that can be measured in the $^1$H-MRS spectrum which are most applicable to stroke are N-acetyl aspartate (NAA) and lactate. NAA is an amino acid of unknown function that is found virtually exclusively in neurons. In conditions associated with neuronal loss, such as stroke or multiple sclerosis, NAA decreases or is lost. There is evidence that NAA will increase again if neuronal injury is reversible. The concentration of NAA can therefore act as a marker of functional neurons. NAA concentration may provide a useful measure of residual neuronal activity in the core of an infarct and hence give an indication of the severity of ischemia and the potential for recovery.
Lactic acid is a by-product of anaerobic respiration and is only found in the brain in significant concentrations after cerebral ischemia. It has been suggested, on the basis of previous animal work, that patients with large and persistently elevated concentrations of cerebral lactate ultimately suffer significant neurological impairment and long-term disability, whereas patients with lower concentrations have more benign courses.11

We have previously shown that the volume of an ischemic cerebral infarct, measured on T2-weighted images, is related to patient outcome in MCA infarcts.12 The purpose of this study was to determine whether the initial metabolite concentrations, measured within the center of an infarct by 1H MRS, could be used to improve the prediction of outcome in patients with middle cerebral artery (MCA) territory infarcts.

**Subjects and Methods**

Thirty-one patients (mean age 66.9 years; range 28 to 90 years) presenting within 72 hours of the onset of an acute, cortical, MCA territory infarct were studied. Patients with lacunar syndromes were excluded. For those who had a stroke while asleep, time from onset was calculated from the time they were last known to be well. Table 1 shows the characteristics of these patients. Patients who had a history of previous stroke were excluded, but those in whom minor abnormalities possibly attributable to vascular disease (eg, mild leukoaraiosis) were found on the scan were not excluded. Patients were evaluated by a neurologist before the first MR examination, and the severity of the of clinical deficit was described with use of the Scandinavian Stroke Scale (SSS).13 Patient outcome was graded at 3 months by the same observer, blind to the 1H MRS results but not the imaging findings, by categorizing the patients into 1 of 3 groups: “independent,” “dependent,” or “dead,” as used in the International Stroke Trial.14

All MR examinations were performed on a 1.5-T whole-body system (GE, Signa) using a standard quadrature birdcage head coil. Diagnostic T2-weighted images were obtained (contiguous, 5-mm-thick slices) using the fast spin-echo technique (echo train length 8, TE 95 to 102 ms, TR 3500 ms). Diffusion-weighted imaging was not available on our scanner. Infarct volumes were calculated from the T2-weighted images using the volume estimator algorithm in the ANALYZE image analysis software package, as previously described.12,13 A 2 × 2 × 2-cm voxel within the center of the infarct was usually chosen for 1H MRS. If the infarct was too small or irregularly shaped, the largest voxel that was still within the core of the infarct was selected. In each case, the voxel was placed wholly within the area that appeared abnormal on the T2-weighted image. Shimming of the magnetic field was performed, and then stimulated echo acquisition mode spectroscopy14 was carried out at short echo times (TE 30 ms, TR 2020 ms, and mixing time of 13.7 ms) using the manufacturers’ automated spectroscopy protocol, the proton brain examination.15 Metabolite concentrations were calculated using variable projection time domain fitting analysis13 after the residual water signal had been removed using the Hankel Lanczos singular value decomposition method.16,17 Resonance peaks were assigned with creatine at 3.94 ppm, choline at 3.22 ppm, NAA at 2.01 ppm, and the lactate doublet at 1.33 ppm with a 7-Hz splitting. The water resonance at 4.7 ppm was used as the internal standard, assuming a concentration of 41.7 mol/L.21 No correction for T1 or T2 relaxation was made.22 The time taken to examine a patient with the protocol was approximately 45 minutes on each occasion.

Comparison of infarct volume and metabolite concentration in the outcome groups was done with ANOVA. Infarct volume had a skew distribution and was therefore log transformed. After a positive F test, Gabriel’s multiple comparison test for unequal groups was used. The Kruskall-Wallis 1-way ANOVA was used to compare initial SSS score in each outcome group, followed by a multiple Mann-Whitney test. Relationships between continuous variables were examined with the product moment correlation coefficient. Confidence intervals for proportions were obtained by the exact binomial method and 2-way tables analyzed by the Fisher exact test. Logistic regression analysis with NAA concentration and log volume as the dependent variable, blind to the SSS score and the actual outcomes, were compared using the k statistic. It was not possible to produce positive predictive values for the combination of infarct volume and NAA concentration with outcome because the cutoff points between high and low values were calculated retrospectively.

**Results**

Figure 1 shows a typical T2-weighted image and 1H MR spectrum obtained in this study. Between the 3 outcome groups, there was no significant difference of the mean age of

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Independent</th>
<th>Dependent</th>
<th>Dead</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61±9</td>
<td>69±16</td>
<td>70±12</td>
<td>0.3</td>
</tr>
<tr>
<td>Time to scan, h</td>
<td>25.7±18.6</td>
<td>34.3±15.6</td>
<td>24.8±15.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Infarct volume, mL</td>
<td>27.8±22.2</td>
<td>104.9±112.4</td>
<td>134.4±93.0</td>
<td>0.01*</td>
</tr>
<tr>
<td>Initial NAA, mmol/L</td>
<td>8.9±3.9</td>
<td>2.8±3.5</td>
<td>4.2±4.2</td>
<td>0.005†</td>
</tr>
<tr>
<td>SSS score</td>
<td>20±3.5</td>
<td>14.5±7.2</td>
<td>8±5.5</td>
<td>0.02‡</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>16.5±7.4</td>
<td>21.4±9.9</td>
<td>24.3±11.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Choline, mmol/L</td>
<td>1.5±0.4</td>
<td>1.2±0.6</td>
<td>1.4±0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Creatinine, mmol/L</td>
<td>7.3±3.1</td>
<td>4.6±2.5</td>
<td>5.1±3.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Mean ± SD values are given for age, time to scan, infarct volume, initial NAA, lactate, choline, and creatinine in the 3 outcome groups; median and interquartile range is given for the SSS. The means or mean ranks in the 3 outcome groups were compared with ANOVA, followed by Gabriel’s test if a significant difference was obtained, or the Kruskal-Wallis test for the SSS.

*Significantly lower initial infarct volume in the independent group compared with the other 2 outcome groups (\( P<0.05\)).
†Significantly higher NAA concentration in the independent group compared with the other 2 outcome groups (\( P<0.01\)).
‡Significantly higher SSS score in the independent groups compared with the other 2 outcome groups (\( P<0.01\)).
the patients or the time interval between symptom onset and the initial examination (Table 1). Patients who had a good outcome at 3 months were, on average, significantly less disabled when evaluated with the SSS before the MR examination than the patients who eventually died or were left disabled ($P<0.01$, Figure 2). However, there was no significant difference in the SSS scores between the dead or dependent groups. Similarly, regarding infarct size, patients who did well at 3 months on average had smaller infarcts than those who did poorly, and this difference was significant ($P<0.05$, Figure 3). There was, however, no significant difference in the mean size of the infarct between those who died and those who were dependent at 3 months.

$^1$H MRS showed no significant association between the concentrations of creatine, choline, or lactate with patient outcome (Table 1). However, there was an association between initial NAA concentration and clinical outcome (Figure 4). Patients who were independent at 3 months had a significantly higher NAA concentration than those who did poorly ($P<0.01$). There was no difference between the NAA concentration in the dead and the dependent groups. There was a correlation between the initial infarct size, log(volume), and the initial NAA concentration ($r=-0.64$, $P=0.0001$).

Because there was no significant difference of the measured parameters between the patients in the dead and dependent groups, these groups were amalgamated to enable all the patients who had a poor outcome to be compared with those who had a good outcome. The measurements of infarct volume and NAA concentration were divided into high and low values. All patients who had an infarct $>70$ mL did poorly (100%; 95% CI 75% to 100%). Therefore, the threshold for large infarct volume was placed at 70 mL. The relationship between NAA and outcome was not so clear cut, but 18 of 20 patients (90%; 95% CI 68% to 99%) with a core NAA concentration $<5.2$ mmol/L did poorly at 3 months whereas 7 of 11 patients (64%; 95% CI 31% to 89%) with an initial NAA concentration $>7.7$ mmol/L did well. This difference was highly significant ($P=0.007$, 2-sided Fisher exact test). Although this test is data dependent and must be treated with some caution, it does provide an indication that there may be a threshold effect of NAA concentration on outcome. There were no patients with NAA in the range 5.2 to 5.7.
to 7.7 mmol/L. Therefore, an arbitrary threshold value of 7 mmol/L was chosen. The result of combining NAA concentration, categorized in this way, with infarct volume is shown in Figure 5. All patients who had an initial infarct volume >70 mL did poorly irrespective of the NAA concentration. Of the patients with small infarcts, those who had a high NAA concentration did well, whereas those with a low concentration of NAA did poorly. There were 3 exceptions to this association. Two patients with small infarcts (54 and 42 mL, respectively) recovered well despite having a low initial NAA. Both had infarcts in the posterior parietal region, which spared the motor and sensory cortex. The location of these infarcts probably explains why they recovered well. One patient had a small infarct and high initial NAA but remained dependent at 3 months. This patient suffered further ischemic events during the study period, which account for his poor recovery.

Initial infarct volume of <70 mL alone predicted good clinical outcome with sensitivity of 100% but specificity of 59%. The corresponding values for NAA alone, measured to be >7 mmol/L in the core, were 78% and 82%, respectively. However, if both measurements were used, the sensitivity to predict a good outcome was 78%, with a specificity of 95%. SSS predicted good outcome with 100% sensitivity and 36% specificity, assuming that the cutoff score to predict a good outcome was 15. Logistic regression analysis using log(volume) and NAA concentration as predictors was used to obtain an equation of the log odds of a good outcome. Log(volume) was used because the distribution was skewed, whereas NAA appeared to have an approximately normal distribution. The overall regression was highly significant: $\chi^2=11.41$, $P=0.003$. However, because log(volume) and NAA were correlated ($r=-0.64$, $P=0.0001$), neither term in the regression was significant by itself. The odds of good outcome decreased by a factor of 0.63 for doubling of the volume and increased by 1.26 for each unit increase in NAA. The actual equation was as follows: log odds of good outcome = $0.15 - 0.66 \times \log(\text{volume}) + 0.23 \times \text{NAA concentration}$.

If this equation was used to predict the more likely outcome, there was moderate agreement between the observed and predicted outcome ($\kappa=0.49$, $P=0.0025$; Table 2).

**Discussion**

The main finding in this study is that the combination of MRS with MRI predicted the outcome in patients with MCA territory infarction, which suggests that the combination can be used to assess the severity of cerebral ischemia directly. Measuring infarct volume allowed the identification of patients with infarcts >70 mL in volume who made a poor recovery from their stroke, whatever the severity of ischemia within the core. Therefore, large infarct volume by itself is a good predictor of poor outcome. Volume alone does not allow the separation of patients with infarcts <70 mL into good or poor prognostic groups. MR spectroscopy can aid prediction of outcome in this latter group of patients; patients with a residual NAA concentration >7 mmol/L did well. Patients with a core NAA concentration below this value tended to be either dead or dependent at 3 months.

Measurement of the NAA concentration provides objective and quantitative information about the biochemical function of neurons remaining in the core of the infarct and can be

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**Figure 4.** Association between initial NAA concentration in the center of the infarct and clinical outcome at 3 months. Squares and error bars represent the means and SDs.

**Figure 5.** Venn diagram showing the relationship between the initial infarct volume, NAA concentration, and clinical outcome at 3 months. Open circles represent patients who were independent at 3 months; filled circles, patients who were either dead or dependent at 3 months. Dashed circles represent measurement of initial infarct volume: the upper circle represents patients with an initial infarct volume >70 mL and the lower circle those with an initial volume <70 mL. Solid circles represent initial NAA concentration measured in the center of the infarct: the left circle represents patients with an initial NAA concentration of <7 mmol/L and the right circle those with an NAA concentration >7 mmol/L.

**TABLE 2.** Comparison of the Predicted Clinical Outcomes, Calculated From Logistic Regression Analysis, to the Actual Clinical Outcome

<table>
<thead>
<tr>
<th></th>
<th>Predicted Bad Outcome</th>
<th>Predicted Good Outcome</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed bad outcome</td>
<td>20</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>Observed good outcome</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>7</td>
<td>31</td>
</tr>
</tbody>
</table>

A predicted outcome was deemed to be the more likely if the probability of its occurring was 0.5. The expected agreement was 80.7% and the actual agreement was 61.5%; $\kappa=0.49$ ($P=0.0025$).
used to monitor these changes over time. Our results confirm that 1H MRS provides a method for assessing the severity of cerebral infarction at presentation and at follow-up. These results are consistent with the hypothesis that NAA concentration reflects the depth of ischemic neuronal damage. Patients with a low NAA concentration made a poor recovery whereas patients with a higher core NAA concentration were more likely to recover fully, which indicated that they had less-severe ischemia and a greater amount of viable neuronal tissue. Our study confirms a report by Ford et al., who studied a small cohort of patients over time and found that the patients who did well had relatively normal levels of NAA. Our findings are consistent with those of Wardlaw et al., who also reported that clinical outcome was related to extent of infarction and reduction in blood flow and that reduction in NAA concentration was related to reduction in blood flow.

Logistic regression analysis confirmed that the prognosis could be predicted from a combination of NAA and initial infarct volume (Table 2), but these results need to be confirmed by further studies. It was not surprising that there was a correlation between NAA concentration and infarct volume, because a larger infarct volume may have a lower core blood flow and hence lower NAA concentration. However, because the overall regression was highly significant, this suggests that these 2 parameters exerted separate pathological effects related to outcome.

This study focused on the changes in the center of the infarct. The finding that the NAA concentration, measured in a small region in the center of the infarct, is related to the eventual clinical outcome suggests that the NAA concentration measured in the center of the infarct is an indicator of the severity of neuronal loss within the whole region of infarction. It is known that the core NAA concentration in some patients continues to decline after the onset of stroke. Therefore, if the patients who had a poor clinical outcome were examined later than those who had a good clinical outcome, the lower NAA concentration might be related to the timing of the examination rather than the severity of neuronal loss. However, in our study there was no significant difference in the timing between the 2 groups (Table 1), which suggests that this phenomenon has not influenced our results.

There has been much interest in the literature about the role of lactate in cerebral ischemia, and the pathogenesis of cerebral infarction has been extensively studied in animals. Lactate production results from the anaerobic metabolism of glucose during ischemia. In animals, the degree and extent of tissue damage has been correlated with the level of lactic acid. In humans, it has been shown that lactate concentrations in the infarct, determined by 1H-MRS, correlated with outcome, but the cohort of patients studied included patients with lacunar and cortical infarcts, and the prognoses of these subtypes of stroke are very different. Furthermore, a small study (6 patients with MCA territory infarction) by Gideon et al. found no relationship between infarct lactate and outcome. We, also, did not find a correlation between lactate and outcome. However, in animal experiments, the initial lactate is measured within a few minutes of the induction of experimental ischemia, whereas in our patients the MR examination was performed later, allowing a variable amount of lactate to be cleared from the ischemic region either by diffusion or removal by collateral blood flow or recanalization of the blood vessels. This may explain why our results differ from those in animals. None of the other metabolite concentrations in the proton spectrum could be correlated to patient outcome.

There are several clinical rating scales available designed to assess the severity of stroke that also predict clinical outcome. We selected the SSS because it is a very simple outcome scale but has sufficient detail to give a good description of the stroke patient. In general, the SSS indicates the volume of brain affected by ischemia, because larger infarcts will inevitably cause more extensive impairment of eloquent regions of the cerebral cortex and a lower SSS score. However, our results suggest that within each SSS category (eg, upper limb power), the degree of paresis reflects the severity of neuronal loss in that region, not smaller area of infarction.

Clinical scales are widely used for describing patients in clinical trials. However, they are cumbersome and operator dependent, particularly among the junior medical and nursing staff members who assess patients initially. MR imaging and spectroscopy provides a noninvasive and effective method for directly studying cerebral infarction. The extent of involvement and the severity can be evaluated directly. This may prove more useful for selecting subjects for clinical trials of treatment in stroke. It may be better to recruit patients with small (<70 mL) infarcts into treatment trials, because they would have a better chance of showing a treatment benefit. It may be possible to use MR spectroscopy to follow the course of infarction, a fall in NAA suggesting further neuronal impairment. Because each 1H MRS examination adds only 15 minutes to a standard brain MRI and provides much more information about the severity of ischemia, 1H MRS may have a useful role in the assessment of stroke patients.

Acknowledgments
We would like to thank the Stroke Association for funding this research. Dr Howe and Prof Griffith acknowledge the support of the Cancer Research Campaign.

References


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*Stroke*. 1999;30:1577-1582
doi: 10.1161/01.STR.30.8.1577

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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