Chapter 4

N-Acetyl-Aspartate: Serum marker of reperfusion in ischemic stroke

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Abstract

Objective: Conventional ways of monitoring reperfusion in acute ischemic stroke have several limitations. In searching for an alternative, we evaluated if biochemical serum markers of stroke change in relation to reperfusion.

Methods: N-Acetyl-Aspartate (NAA) is a small amino acid synthesized by neuronal mitochondria, which can be released in the extracellular space following reperfusion in animal models of brain ischemia. S-100B is a well known peripheral marker of brain damage in various neurological diseases, including stroke. Serum samples were analyzed from 13 patients with ischemic stroke who were either treated conservatively or with recombinant tissue plasminogen activator (rtPA). Blood was drawn at baseline, after 30 minutes, after 1, 2, 4, 8 hours, and between 12-24 hours. Serum concentrations of NAA were analyzed using a gas chromatography-mass spectrometry method. S-100B was analyzed using an automated immunoluminometric assay. Reperfusion was assessed using transcranial doppler (TCD) and clinical criteria.

Results: Reperfusion (n=4) was associated with a transient rapid increase in serum NAA levels. Such an early rapid increase of NAA was not observed in patients with persistent occlusion at 12-24 hours (n=4) and patients with no occlusion on baseline TCD (n=5). NAA peak levels and AUC values were significantly higher after reperfusion in comparison with normal TCD findings or persistent occlusion (p=0.003 and p=0.05 respectively). No differences were found between these groups for S-100B levels.

Conclusions: In patients with acute ischemic stroke serum NAA levels transiently raise following early reperfusion.
**Introduction**

Reperfusion is an important goal in acute ischemic stroke therapy. Several imaging and ultrasound techniques exist for vascular monitoring during or after thrombolytic therapy. Repeated imaging procedures are often not practical in acute stroke care, but several studies have shown the feasibility of TCD as a recanalization monitoring tool. However, some patients do not have sufficient temporal bone windows and distal vessel occlusions may not be detected. In this study, we explored if biochemical serum markers of brain injury can offer an alternative way of monitoring reperfusion.

N-Acetyl-Aspartate (NAA) is a small amino acid present in high concentrations in the brain. It is synthesized and stored in neurons, but catabolism is regulated by oligodendrocytes. Although its exact function is largely unknown, synthesis is directly coupled to glucose metabolism, and it may have a role in osmoregulatory mechanisms. In proton nuclear magnetic resonance spectroscopy (MRS) studies, NAA can be detected and is regarded as a marker of neuronal function and integrity. In patients with ischemic stroke, MRS has shown reductions of NAA in the infarct region. We are only aware of one study that measured serum NAA levels in stroke patients. This study suggested a correlation between infarct size and NAA levels in patients with a Glasgow Outcome Score (GOS) < 5. Interestingly, microdialysis experiments in striatum of rodents following transient global or focal brain ischemia found that reperfusion induced a transient release of NAA in the extracellular space. S-100B is not only a peripheral marker of brain damage in various neurological diseases, but is probably also released after damage to the blood-brain barrier. In patients with acute ischemic stroke, serum levels of these proteins correlate with infarct size, and with neurological and functional outcome. One study showed that low S-100B values obtained 48-96 hours after stroke onset indicated successful clot lysis with high accuracy. Although such findings may be used as secondary end points in large-scale thrombolytic studies, they cannot guide acute stroke therapy decisions. Therefore, serum markers must change rapidly in concentration in relation to reperfusion to be clinically useful. Frequent sampling before, during and after therapy would be necessary to identify such serum markers.

In the present study, our aim was to investigate whether reperfusion in patients with acute ischemic stroke induces early changes in serum NAA and S-100 levels.

**Methods**

**Patients**

The study was approved by the local ethics committee. The study population consisted of patients with presumed partial or total anterior circulation ischemic stroke, aged 18 years or
older, and known stroke onset time. Minimal stroke severity for inclusion was a NIHSS score of at least 2. A Computed Tomography (CT) scan of the brain was performed in all patients at baseline. Exclusion criteria included cerebral hemorrhage, suggested lacunar stroke, significant pre-stroke handicap defined as a Rankin score of 3 or higher, and severe concomitant disease likely to influence clinical assessment during the study.

**NAA and S-100B**

Venous blood samples were taken at baseline within 5 hours after stroke onset. In patients receiving rtPA the first sample was taken just before administration of rtPA. Further samples were taken after 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and between 12 and 24 hours. NAA was analyzed using a modification of a stable isotope dilution gas chromatography mass-spectrometry (GC-MS) method, which has been used previously by Stevens et al. This method has also been used for analysis of NAA in several body fluids for the diagnosis of Canavans disease. Reference values for NAA were calculated from a population of 28 normal subjects (12 females, mean age +/-SD ; 44 +/- 9 years). S-100B was analyzed using monoclonal sandwich immunoluminometric assays (Sangtec, Bromma, Sweden) with a fully automated system.

**TCD measurements**

TCD examinations were done using a 2-MHz transducer system (Pioneer WinTCD Version 2.0, Nicolet Biomedical Inc., Madison, WI, USA) and middle cerebral artery (MCA) flow signals were evaluated in the distal MCA segment at depths of 40-54 mm. TCD was performed during the 1 hour infusion of rtPA in the emergency department, or the first hour after baseline sampling for control patients. When abnormalities persisted at the end of this period, repeat TCD was performed between 12 and 24 hours after the baseline sample. TCD results were evaluated using the Trombolysis in Brain Ischemia (TIBI) system, which uses 6 grades: grade 0-1 minimal or absent flow, grade 2-3: dampened or blunted flow signals and grade 4-5: stenotic or normal MCA flow signals. Reperfusion was defined as an improvement from an initial grade 0-1 or grade 2-3 residual flow signal to a grade 4 or 5 signal, or as an initial grade 0-1 or grade 2-3 residual flow signal followed by early neurological recovery on the NIHSS (see neurological assessment). Persistent occlusion was defined as persisting grade 0-1 or grade 2-3 signals.

**Neurological assessment**

Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS). As clinical measure for reperfusion we monitored neurological recovery. Early neurological recovery was defined as an improvement on the NIHSS of 10 points or more or complete recovery at 24 hours.
Statistics

To analyze S-100B and NAA data, the area under the curve (AUC) and the individual peak levels were used as summary measures. Mann Whitney U tests were performed to compare the S-100B and NAA data between groups, using exact statistics because of the small sample size. Baseline NIHSS scores were compared using the Kruskall Wallis test.

Results

Patients

In all, 16 patients were screened for inclusion in this study. Three patients were excluded because temporal bone windows were insufficient for an adequate TCD assessment. CT scanning at baseline was consistent with ischemic stroke in all patients. On the initial TCD, there were 6 patients with TIBI grade 0 or 1; 2 with TIBI grade 3; and 5 with TIBI grade 4-5. Patients were grouped into three categories. Group 1 included those with permanent MCA occlusion on follow up TCD done between 12-36 hours after stroke onset (n=4). Group 2 included those with stenotic (n=1) or normal (n=4) signals on initial TCD. Group 3 included those with reperfusion (n=4), two of whom demonstrated such during rtPA infusion on TCD, and two with initial complete or partial MCA occlusion on TCD, with early clinical recovery (>= 10 NIHSS points), but without TCD evidence. Follow-up TCD could not be performed in these 2 patients because 1 patient died of intracranial hemorrhage, and 1 developed agitated delirium. Four patients demonstrated early recovery at 24 hours, 3 in group 3 and 1 in group 2. Figure 1 shows the mean NIHSS scores for all groups.

![Figure 1. NIHSS scores (mean +/- SEM) for the 3 groups.](image)

Baseline NIHSS scores were not different between the groups (p=0.45). One patient of group 3 died on day 2 of intracerebral hemorrhage in the infarct region with intraventricular hemorrhage after early improvement (12 points on the NIHSS) had occurred. In this patient
the last available NIHSS was used on other time points (last observation carried forward). Demographic and baseline characteristics of the 3 groups are shown in Table 1. Four patients were not treated with rtPA on the basis of exclusion criteria.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (persistent occlusion)</th>
<th>Group 2 (normal baseline TCD)</th>
<th>Group 3 (reperfusion)</th>
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<tr>
<td>No. of patients</td>
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<td>203 +/- 70</td>
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<td>2</td>
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Table 1. Demographic and clinical data.

NAA and S-100B
Individual curves of NAA and S-100B for the different groups are shown in figure 2. For NAA, different patterns are visible in the 3 groups. In all patients of group 3, reperfusion was associated with a rapid transient increase in NAA serum levels. Patients of group 1 with persistent occlusion showed a slowly increasing and decreasing pattern, with peak levels around 4-8 hours after baseline. Patients of group 2 with grade 4 or 5 baseline TCD findings had normal or only slightly elevated levels with little variation between the different samples. Peak levels of NAA were significantly higher in patients with reperfusion (group 3) than in those without reperfusion (group 1 and 2)(mean +/- SD 1,08 +/- 0,65 μmol/l vs 0,44 +/- 0,06 μmol/l; p=0,003). AUC values were also significantly higher (mean +/- SD 15,0 +/- 8,8 μmol/l × h vs 8,7 +/- 1,5 μmol/l × h; p=0,050). In all groups S-100B values remained normal or slightly elevated over the first 4-8 hours. Peak levels were usually observed at 12-24 hours,
but were not always increased above normal. Peak levels and AUC values of S-100B were not different between groups (respectively 0.73 +/- 0.68 ng/ml vs 0.60 +/- 0.60 ng/ml; p=0.74 and 13.5 +/- 11.4 ng/ml × h vs 7.2 +/- 5.4 ng/ml × h; p=0.33).

**Figure 2.** Individual curves for NAA and S-100B for all patients in all groups. Note the change of scale in the Y-axis of some of the figures. Thick horizontal lines: upper limit of normal values. B: baseline sample. Arrows: moment of reperfusion as detected by TCD. Dotted lines: reperfusion based on early neurological recovery.

**Discussion**

In this study, we have shown that reperfusion following acute ischemic stroke is associated with a transient increase in serum NAA levels. This was not observed for serum S-100B levels. One earlier study reported that serum NAA was elevated early in the course of ischemic stroke and might correlate with infarct size in patients with a GOS score < 5. It was not the purpose of the present study to correlate serum NAA levels with outcome, but it is of interest to note that in this earlier study, the highest serum levels of NAA were found in two patients who had the best clinical outcome and smallest infarct size, which rather suggests that NAA could be released in large amounts from reversibly affected neurons. This has also
been demonstrated in animal studies\textsuperscript{16}. Furthermore, microdialysis studies have shown that during early ischemia extracellular NAA levels in the central nervous system increase rapidly, with a more than 10 fold increase after reperfusion, and subsequent normalization after 45 minutes\textsuperscript{8}. The serum NAA results of our study are similar to the time course of these interstitial NAA changes. Thus, the temporal course of serum NAA changes is rapid, which can also explain the lack of changes in serum NAA in patients with grade 4 or 5 findings on initial TCD. In some of these patients, spontaneous reperfusion could have occurred before baseline sampling, and any increases in serum NAA may have been missed. Therefore, increased serum NAA levels after reperfusion probably reflect washout of interstitial NAA through a defective blood-brain barrier. It has been proposed that NAA could function as an efflux metabolic water pump for the removal of neuronal metabolic water. It has been calculated that 1 mol of NAA is synthesized for every 40 mol of glucose equivalent oxidized in the brain, and each mol of NAA may transport 121 mol of metabolic water out of neurons\textsuperscript{5}. Enhanced neuronal production and release of NAA after reperfusion might be caused by the restoration of energy and serve to remove neuronal water that has accumulated during ischemia.

To accurately assess the value of NAA monitoring in acute ischemic stroke further studies are needed. If a consistent relation between the moment of reperfusion and increase in serum NAA levels can be established, serum NAA levels could be useful for monitoring reperfusion in cases where TCD is not possible or available. However, the analytical method would have to be improved because the current method is too time consuming to be used in an emergency setting.

References


