

Neurobiochemical Markers of Brain Damage in Cerebrospinal Fluid of Acute Ischemic Stroke Patients

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BACKGROUND: Ischemic injury to the central nervous system causes cellular activation and disintegration, leading to release of cell-type-specific proteins into the cerebrospinal fluid (CSF). We investigated CSF concentrations of myelin basic protein (MBP), glial fibrillary astrocytic protein (GFAP), the calcium-binding protein S100B, and neuron-specific enolase (NSE) in acute ischemic stroke patients and their relation to initial stroke severity, stroke location, and long-term stroke outcome.

METHODS: CSF concentrations of MBP, GFAP, S100B, and NSE were assessed in 89 stroke patients on admission (mean 8.7 h after stroke onset) and in 35 controls. We evaluated the relation between CSF concentrations and (a) stroke severity (NIH Stroke Scale [NIHSS] score on admission, infarct volume), (b) stroke location, and (c) stroke outcome (modified Rankin Scale [mRS] score at month 3).

RESULTS: MBP concentration was significantly higher in subcortical than in cortical infarcts (median MBP, 1.18 vs 0.66 $\mu\text{g/L}$, $P < 0.001$). GFAP and S100B concentrations correlated with the NIHSS score on admission (GFAP, $R = 0.35$, $P = 0.001$; S100B, $R = 0.29$, $P = 0.006$), infarct volume (GFAP, $R = 0.34$, $P = 0.001$; S100B, $R = 0.28$, $P = 0.008$), and mRS score at month 3 ($R = 0.42$, $P < 0.001$ and $R = 0.28$, $P = 0.007$). Concentrations of NSE did not correlate with stroke characteristics.

CONCLUSIONS: MBP, GFAP, S100B, and NSE display relevant differences in cellular and subcellular origins, which are reflected in their relation to stroke character-

istics. MBP is a marker for infarct location. GFAP and S100B correlate with stroke severity and outcome.

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During the last decade, neurobiochemical markers in stroke patients have attracted increased attention (1, 2). Ischemic injury to the central nervous system causes cellular activation and disintegration, leading to release of cell-type-specific proteins such as myelin basic protein (MBP),⁸ glial fibrillary astrocytic protein (GFAP), the calcium-binding protein S100B, and neuron-specific enolase (NSE). Measurable amounts of these damage markers are present in cerebrospinal fluid (CSF) and blood. The relation between serum and CSF concentrations is poor, however, a situation attributed to relevant confounding factors for brain markers in blood (3–7). Yet, despite the fact that CSF concentrations more accurately reflect cerebral pathological changes, current knowledge about CSF concentrations of brain damage markers in ischemic stroke is based on a small number of studies (6, 8–10).

These damage markers have their own unique biochemical background and display relevant differences in cellular and subcellular origins. MBP is a myelin membrane proteolipid that is bound to cellular membranes of central myelin and, to a lesser extent, of peripheral myelin (11). GFAP is a structural protein expressed almost exclusively in astrocytes and released upon cellular disintegration and degradation of the cytoskeleton (12). The calcium-binding protein S100B is mainly expressed in astrocytes but is also present in oligodendrocytes, microglia, neurons, and extracerebral tissue (13–15). In addition to its immediate release from dying cells, S100B is also actively secreted in a

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⁸ Nonstandard abbreviations: MBP, myelin basic protein; GFAP, glial fibrillary astrocytic protein; NSE, neuron-specific enolase; CSF, cerebrospinal fluid; TIA, transient ischemic attack; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

Table 1. Patient and stroke characteristics of 89 patients with hyperacute ischemic stroke or TIA and 35 controls.^a

Characteristic	Value		
	Stroke or TIA (n = 89)	Controls (n = 35)	P value ^b
Patient characteristics			
Age, years	71.1 (13.2)	68.1 (12.5)	0.26
Male sex	50 (56.2%)	18 (56.3%)	0.54
White	87 (97.8%)	31 (96.9%)	0.97
Time to sampling, h	8.7 (6.2)	NA	
Stroke characteristics			
TIA/ischemic stroke	21 (23.6%)/68 (76.4%)	NA	
NIHSS on admission	4 (1–11)	NA	
Infarct volume, mL	4.9 (1.2–56.5)	NA	
mRS at month 3		NA	
mRS 0–3	69 (77.5%)		
mRS 4–6	20 (22.5%)		
Stroke etiology ^c			
Cardioembolic	39 (43.8%)		
Lacunar	20 (22.5%)		
Atherothrombotic	21 (23.6%)		
Specific	3 (3.4%)		
Undetermined	6 (6.7%)		

^a Data are given as mean (SD), number (percentage), or median (interquartile range). NA, not applicable.
^b t-Test for continuous variables, cross-tabulations and χ^2 -test for categorical variables.
^c Stroke etiology classified by the TOAST criteria.

regulated manner, independent of cell death (16). NSE is present in neuronal cytoplasm and insignificant quantities of this protein are found in neuroendocrine cells (17, 18).

Given the paucity of available data in the literature, we investigated in this study the CSF concentrations of MBP, GFAP, S100B, and NSE in acute ischemic stroke and their relation to initial stroke severity, stroke location, and long-term stroke outcome.

Materials and Methods

STUDY POPULATION

This study is part of the Middelheim's Interdisciplinary Stroke Study, which is a project on the clinical, biochemical, neuroimaging, neuropsychological, and electrophysiological evaluation of patients with ischemic stroke or transient ischemic attack (TIA) at ZNA Middelheim Hospital, Antwerp. Other biochemical analyses from this project have been reported elsewhere (19–23). In this study, we focused on a cohort of 89 patients with ischemic stroke (n = 68) or TIA (n =

21) in whom analysis of brain damage markers in CSF was available. Lumbar puncture was performed on admission (mean [SD] 8.7 [6.2] h after onset of stroke symptoms). The study was conducted according to the revised Declaration of Helsinki (1998) and in agreement with the guidelines of the ethics committees of ZNA Antwerp and the University of Antwerp. Patient and stroke characteristics of the study population are shown in Table 1.

CONTROL POPULATION

Because data on concentration reference intervals for MBP, GFAP, S100B, and NSE in CSF are scanty and vary according to the applied technique, we included a control population consisting of 35 individuals without antecedents of central nervous system disease and without any contraindication for lumbar puncture. Indications for lumbar puncture included investigation of peripheral nervous system disorders (n = 25), suspicion of meningitis (n = 2), suspicion of subarachnoid hemorrhage (n = 3), and subjective memory dys-

function ($n = 2$). Routine analysis for all controls showed CSF results to be within reference intervals.

LUMBAR PUNCTURE AND MEASUREMENT OF BRAIN DAMAGE MARKERS

In total, approximately 15 mL of CSF was collected in polypropylene vials. Uncentrifuged samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. A traumatic tap was recognized by visual inspection and by the “3-tube test.” CSF samples that were not clear or that initially contained blood with gradual clearing were excluded from analysis. Routine investigation of CSF included cell count, total protein, and glucose analysis as well as agar gel electrophoresis of proteins. All CSF samples contained fewer than 5 white cells/ mm^3 . Before analysis samples were defrosted and centrifuged at 1500g for 10 min at 4°C . All samples were blinded to case identity and analyzed in duplicate. MBP, GFAP, S100B, and NSE were quantified by commercially available ELISA kits (Human MBP ELISA, DSL; Human GFAP ELISA, BioVendor; Human S100B ELISA, BioVendor; Human NSE ELISA, DRG Instruments) in accordance with the manufacturer’s instructions. Sample volumes per single assay were 50 μL (MBP), 33.3 μL (GFAP), 25 μL (S100B), and 25 μL (NSE). Analytical limits of quantification were 0.1–9 $\mu\text{g}/\text{L}$, 0.14–25 $\mu\text{g}/\text{L}$, 5–2000 ng/L, and 1–50 $\mu\text{g}/\text{L}$ for the MBP, GFAP, S100B, and NSE ELISA, respectively. The reported interassay CV was 5.2% at 0.402 $\mu\text{g}/\text{L}$ for the GFAP ELISA, 5.2% at 416.9 ng/L for the S100B ELISA, and 5.5% at 10.3 $\mu\text{g}/\text{L}$ for the NSE ELISA. Interassay CVs were not available for the MBP ELISA. Intraassay CVs were 4.3%, 4.4%, 4.9%, and 3.4%, for the MBP, GFAP, S100B, and NSE ELISA, respectively.

EVALUATION OF STROKE SEVERITY, LOCATION, AND OUTCOME

At the time of admission, patient neurological deficits were quantified by trained stroke physicians using the NIH Stroke Scale (NIHSS). Except for 2 patients who died before repeat neuroimaging was performed and 3 patients with a contraindication for MRI, all patients, in addition to baseline neuroimaging on admission, underwent an MRI scan of the brain on average 3.1 days after stroke onset. The patients in whom MRI was contraindicated were evaluated by computed tomography of the brain on average 3.0 days after stroke onset. The infarct location and volume were assessed by 2 independent observers as described previously (19–23). Acute cerebral ischemia confirmed by computed tomography or MRI of the brain was found in 58 patients, and the median infarct volume was 4.9 mL (interquartile range, 1.2–56.5 mL). Outcome was assessed at 3 months after stroke by means of the modi-

fied Rankin Scale (mRS). In agreement with the literature, poor outcome was defined as mRS score >3 (24).

STATISTICAL ANALYSES

Statistical computations were performed with SPSS software package version 15.0 (SPSS). Data normality was assessed using the Kolmogorov–Smirnov test. Results are presented as mean (SD) or median (interquartile range) as appropriate. The Mann–Whitney U -test was applied to assess the differences in biomarker concentration between 2 groups. The relation between biomarker concentration and parameters for stroke severity, location, and outcome was assessed by bivariate correlations (Spearman’s ρ). Logistic regression analysis was performed to determine factors that could be considered independent predictors for stroke outcome.

Results

CSF CONCENTRATIONS OF MBP, GFAP, S100B, AND NSE

The characteristics of the control group are listed in Table 1. Patients and controls were well matched with regard to demographic characteristics. Data were normally distributed for patient age and the interval between stroke onset and lumbar puncture, whereas the NIHSS score on admission, the infarct volume, and the CSF concentrations of biomarkers were not normally distributed (Kolmogorov–Smirnov test). The median CSF concentrations of MBP, GFAP, S100B, and NSE for patients with ischemic stroke or TIA and for controls are shown in Table 2. MBP and GFAP concentrations in CSF were significantly higher in patients with TIA or stroke than in controls, but S100B and NSE concentrations were similar in both study groups. S100B concentrations were positively correlated with GFAP ($R = 0.55$, $P < 0.001$), NSE ($R = 0.53$, $P < 0.001$), and MBP ($R = 0.29$, $P = .006$), but no other correlations were found. CSF biomarker concentrations were not associated with stroke etiology, as assessed by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification (data not shown).

CSF CONCENTRATIONS OF MBP, GFAP, S100B, AND NSE IN RELATION TO STROKE SEVERITY

MBP and NSE concentrations in CSF did not correlate with the NIHSS score on admission or with infarct volume (MBP, $R = 0.01$, $P = 0.955$ and $R = 0.10$, $P = 0.359$; NSE, $R = 0.05$, $P = 0.624$ and $R = 0.14$, $P = 0.20$). GFAP and S100B concentrations, on the other hand, correlated positively with the NIHSS score (GFAP, $R = 0.35$, $P = 0.001$; S100B, $R = 0.29$, $P = 0.006$) and infarct volume (GFAP, $R = 0.34$, $P = 0.001$; S100B, $R = 0.28$, $P = 0.008$). Based on the NIHSS score used to assess stroke severity on admission, patients were categorized as mild stroke (NIHSS ≤ 7 , $n = 54$) or

Table 2. CSF concentration of MBP, GFAP, S100B, and NSE in the study corpus of 89 patients with hyperacute ischemic stroke or TIA and in 35 controls.^a

CSF concentration	Value		P value ^b
	Stroke or TIA (n = 89)	Controls (n = 35)	
MBP, $\mu\text{g/L}$	1.04 (0.61–1.29)	0.53 (0.34–0.77)	<0.001
GFAP, $\mu\text{g/L}$	1.01 (0.76–1.66)	0.61 (0.45–1.06)	<0.001
S100B, ng/L	306.7 (238.1–397.7)	375.0 (270.4–443.5)	0.142
NSE, $\mu\text{g/L}$	3.05 (2.35–4.43)	2.67 (2.17–3.80)	0.076

^a Data are given as median (interquartile range).
^b Mann-Whitney *U* test.

moderate to severe stroke patients (NIHSS >7, n = 35) (25). Patients with moderate to severe stroke had significantly higher GFAP and S100B concentrations than patients with mild stroke (median GFAP, 1.51 vs 0.88 $\mu\text{g/L}$, $P = 0.004$; median S100B, 347.5 vs 286.5 ng/L, $P = 0.007$) (Fig. 1A).

Patients with confirmed stroke on imaging had higher median GFAP concentrations in CSF than TIA patients (1.10 vs 0.83 $\mu\text{g/L}$, $P = 0.004$) and tended to have higher median S100B concentrations (327.4 vs 286.6 ng/L, $P = 0.052$), but MBP and NSE concentrations did not differ between these subpopulations ($P = 0.086$ and $P = 0.378$, respectively). The smallest infarct volume causing a detectable increase in GFAP and S100B was only 0.6 mL. Patient infarcts were classified by volume into small infarct (<5 mL, n = 59) and moderate to large infarct (≥ 5 mL, n = 30) (26). GFAP and S100B concentrations in CSF were higher in patients with moderate to large infarcts than in patients with small infarcts (median GFAP; 1.50 vs 0.88 $\mu\text{g/L}$, $P = 0.001$; median S100B, 389.9 vs 289.6 ng/L, $P = 0.001$) (Fig. 1B).

CSF CONCENTRATIONS OF MBP, GFAP, S100B, AND NSE IN RELATION TO STROKE LOCATION

Based on the neuroimaging findings, stroke location was categorized as pure cortical (n = 15), pure subcortical (n = 18), and corticosubcortical stroke (n = 25). MBP concentrations were significantly higher in patients with subcortical involvement (n = 43) than in patients with pure cortical damage (median MBP, 1.18 vs 0.66 $\mu\text{g/L}$, $P < 0.001$) (Fig. 2). Concentrations of GFAP, S100B, and NSE did not differ between cortical and subcortical infarct location ($P > 0.05$).

CSF CONCENTRATIONS OF MBP, GFAP, S100B, AND NSE IN RELATION TO LONG-TERM STROKE OUTCOME

The mRS score at month 3 correlated with GFAP and S100B concentrations on admission ($R = 0.42$, $P < 0.001$ and $R = 0.28$, $P = 0.007$), but not with MBP or

NSE ($P > 0.05$). The outcome at 3 months after the stroke was poor (mRS 4–6) in 20 patients (22.5%) and good to moderate (mRS 0–3) in 69 patients (77.5%). GFAP and S100B concentrations were significantly higher in patients with poor outcome than in patients with good to moderate outcome (median GFAP, 1.64 vs 0.89 $\mu\text{g/L}$, $P < 0.001$; median S100B, 442.7 vs 283.5 ng/L, $P < 0.001$) (Fig. 3).

Stepwise logistic regression analysis using an inclusion criterion of $P < .10$ and including patient characteristics (age, sex), stroke characteristics (NIHSS score on admission, infarct volume, infarct location, stroke etiology) and possible confounding factors (time interval between lumbar puncture and onset of stroke symptoms, traumatic lumbar puncture as assessed by the number of red blood cells/mm³ CSF) as covariates, identified GFAP, S100B, NIHSS score, and patient age as predictors for stroke outcome at month 3. This result may not be surprising because we found highly significant correlations between GFAP, S100B, and NIHSS score. GFAP and S100B also significantly correlated with age ($R = .31$, $P = 0.003$ and $R = .35$, $P = 0.001$).

Discussion

Biochemical markers for the assessment of brain damage have been used for more than 40 years (11, 17). Large numbers of studies evaluated release patterns of damage markers in blood samples, but the interpretation of neurologic injury markers in blood is hampered by many confounding factors, including variable blood-brain barrier passage, clearance rates from blood that are affected by renal or liver failure, and especially for S100B, contribution of extracerebral tissues to blood concentrations (3, 4). Further questions regarding the validity of cerebral damage markers in blood are raised by the absence of a correlation between

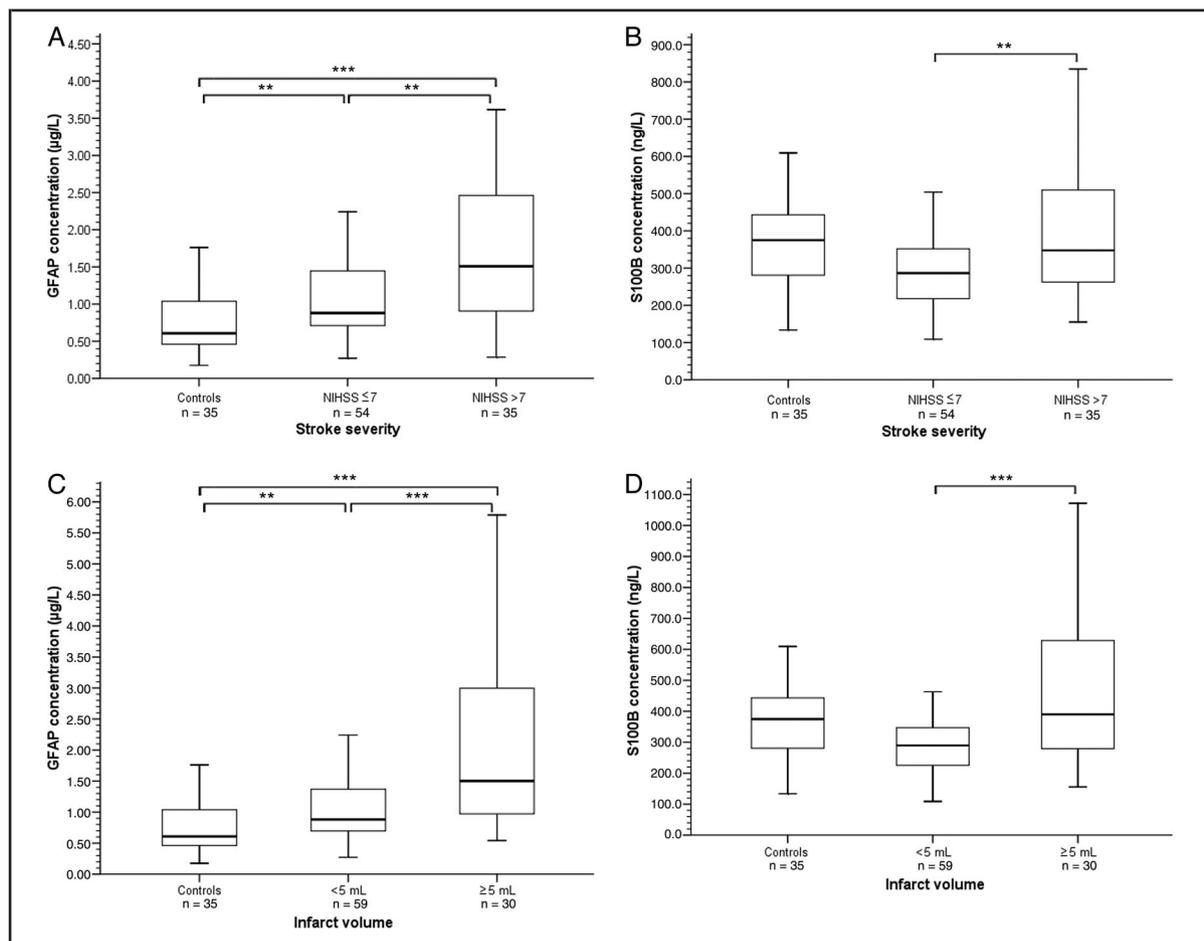


Fig. 1. Box-and-whisker plots demonstrating GFAP and S100B concentrations.

(A,B), GFAP concentrations (A) and S100B concentrations (B) in CSF in patients with mild stroke (NIHSS ≤ 7), in patients with moderate to severe stroke (NIHSS > 7), and in controls. (C,D), GFAP concentrations (C) and S100B concentrations (D) in CSF in patients with small infarct (< 5 mL), patients with moderate to large infarct (≥ 5 mL) and in controls. The black horizontal line in each box represents the median, with the boxes representing the interquartile range. Significant differences are indicated with ** ($P < 0.01$) or *** ($P < 0.001$) (Mann–Whitney U -test).

serum and CSF concentrations in several studies (5–7) and increased serum S100B concentrations in patients without brain injury (27–29). Because CSF is in communication with cerebral extracellular fluid and is less hampered by confounding factors, it is believed that biochemical parameters in CSF more accurately reflect *in cerebro* pathological changes.

To the best of our knowledge, only 4 studies on MBP, GFAP, S100B, or NSE concentration in CSF of ischemic stroke patients have been reported (6, 8–10). All studies involved relatively small study populations, ranging from 28–55 patients, and results may be hampered by relevant methodological drawbacks (such as the absence of a well-matched control population). In previous studies, CSF samples were obtained at various times after stroke onset, but at the earliest, 1–2 days

after onset of neurological symptoms. Except for Aurell et al. (9), who assessed GFAP and S100B, all authors focused on a single damage marker. Measurement of infarct volume was based on computed tomography, apart from the study by Plezold et al. (6), in which T2-weighted MRI was used. Studies investigating other neurobiochemical markers of brain damage in acute ischemic stroke patients identified a marked increase of tau protein in CSF, which correlated with the infarct volume (30), and likewise, tau protein concentrations were found to be increased in ventricular CSF of patients with traumatic brain injury (31). These findings indicate that tau protein concentrations in CSF probably reflect axonal damage. Visinin-like protein-1 also has recently been identified as a promising biomarker for brain injury (32).

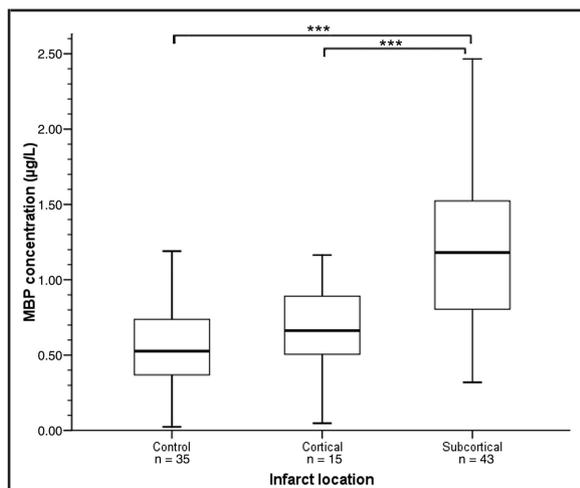


Fig. 2. Box-and-whisker plots demonstrating MBP concentration in CSF in patients with cortical infarct, in patients with subcortical infarct and in controls. The black horizontal line in each box represents the median, with the boxes representing the interquartile range. Significant differences are indicated with *** ($P < .001$) (Mann–Whitney U -test).

This study is the first in which 4 nervous system injury markers were simultaneously evaluated, reflecting damage to all brain compartments (myelin, glia, and neuron) in hyperacute ischemic stroke (mean interval between stroke onset and lumbar puncture was 8.7 h). Our study population represents the entire clinical stroke spectrum, ranging from patients with TIA to patients with severe ischemic stroke. Stroke severity, infarct volume, infarct location, and stroke outcome were evaluated according to a stringent protocol that matches the highest international standards. Our study disclosed that patients with subcortical infarcts display higher CSF concentrations of MBP. In addition, the study showed that concentrations of GFAP and S100B correlate with stroke severity and outcome. Increased MBP concentrations in patients with subcortical infarcts can be attributed to more extensive damage to myelin sheets in the white matter. In contrast to previous findings (10), we failed to find a correlation between MBP CSF concentrations and stroke severity or outcome. This finding might be explained by the fact that MBP primarily reflects white matter damage, and does not indicate injury to gray matter. GFAP release mirrors injury to astrocytes, located both in white and gray matter, and S100B concentration in CSF may increase owing to damage to glial and neuronal cells. It is therefore conceivable that neither GFAP nor S100B concentrations allow differentiation between cortical and subcortical infarct. In line with previous studies

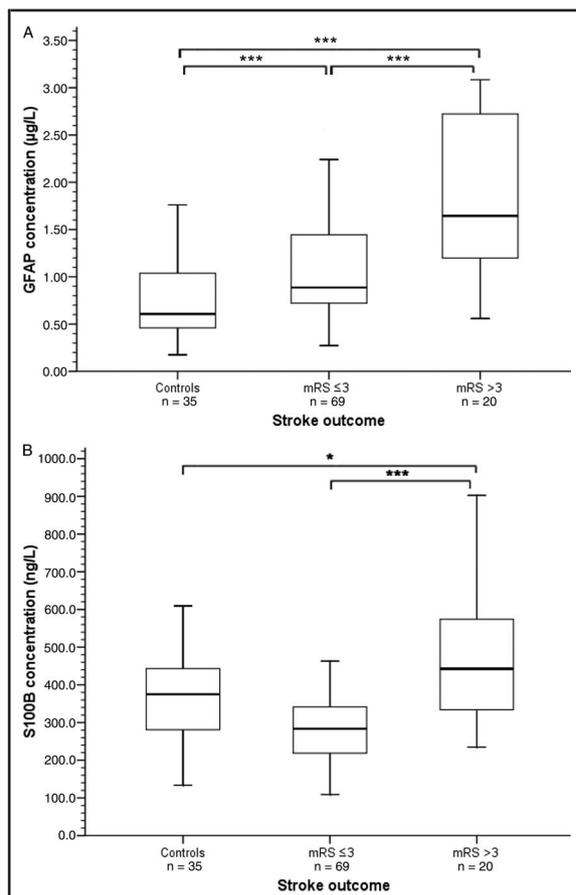


Fig. 3. Box-and-whisker plots demonstrating median GFAP (A) and S100B concentration (B) in CSF in patients with good to moderate outcome (mRS 0–3), in patients with poor outcome (mRS 4–6), and in controls. The black horizontal line in each box represents the median, with the boxes representing the interquartile range. Significant differences are indicated with * ($P < 0.05$) or *** ($P < 0.001$) (Mann–Whitney U -test).

(6, 9) we found that GFAP and S100B concentrations strongly correlate with stroke severity and outcome, likely reflecting more widespread cerebral damage in patients with severe stroke. Logistic regression analysis identified GFAP and S100B concentrations as independent predictors for long-term outcome, but given the collinearity between the biomarkers and the NIHSS score on admission, we cannot exclude that the predictive value of GFAP and S100B concentration is secondary to initial stroke severity. We believe that the lack of a relation between NSE concentrations in CSF and stroke severity, location, or outcome may be due to the very early CSF sampling in our study. Literature data suggest that

NSE concentration in CSF increases significantly only at 24–48 h after onset of cerebral ischemia (8).

Some limitations of this study should be acknowledged when interpreting our data. Only limited data are available regarding CSF concentrations of MBP, GFAP, S100B, and NSE, and current insights on the possible confounding effects of patient characteristics, comorbidity, or concomitant therapy are incomplete. S100B measurement is reported to be particularly susceptible to bias (16, 33). The wide range of S100B CSF concentrations in controls reported in the literature and the rather high median S100B concentration in our control population illustrate this issue. Despite the lack of clinical indications for confounding conditions and normal results of routine CSF assessment in our control population, bias cannot be excluded. In addition, cerebral regional variability of nervous-system-specific proteins and degradation by proteinases may also influence the concentration in CSF. Although the CSF samples contained fewer than 5 white cells/mm³, cellular lysis upon thawing and falsely increased damage marker concentrations, especially of NSE, cannot be excluded. Based on the logistic regression analysis, confounding effects of the patient's age, sex, or traumatic puncture seem rather unlikely.

In summary, the findings of our study add to the insights on the role of biochemical markers for brain damage in acute ischemic stroke. Obviously, CSF sampling has no major place in the diagnostic workup of most ischemic stroke patients, but the findings im-

prove the current knowledge on the pathophysiology of acute ischemic cerebral injury and may help to clarify issues with regard to the use of brain damage markers in blood.

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