

S100B as a surrogate marker for successful clot lysis in hyperacute middle cerebral artery occlusion

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Objectives: The astroglial protein S100B is a marker of cerebral tissue damage. This study investigated whether the serum kinetic of S100B may serve as a surrogate marker of successful clot lysis and early recanalisation (<6 hours) in hyperacute proximal middle cerebral artery (MCA/M1) occlusion.

Methods: The authors prospectively included 23 patients (mean (SD) age, 70.2 (11.0) years) presenting with MCA/M1 occlusion on magnetic resonance angiography (n=18), intra-arterial angiography (IA; n=2), or transcranial Doppler sonography (TCD; n=3) within five hours after symptom onset. Rates of recanalisation and their point of time were determined using TCD or IA. Individual S100B values were determined at hospital admission, every eight hours within the first three days, and at 12 hour intervals from day 4 to day 6. Additionally, the S100B area under the curve (AUC) and the S100B peak value were obtained.

Results: Early recanalisation (<6 hours after symptom onset, n=7) was associated with a significantly lower mean S100B AUC compared with no recanalisation (22.2 (40.1) versus 406.8 (284.4) µg/l per hour; p<0.001). Using receiver operating calculations, a single S100B value obtained 48–96 hours after stroke onset of less than 0.4 µg/l (cut off point) provided a 86% sensitivity and 100% specificity for sufficient MCA/M1 clot lysis <6 hours. The overall accuracy for a single S100B value obtained in the 48–96 hours time window was as high as for the AUC (95.7%).

Conclusion: A single S100B value <0.4 µg/l obtained 48–96 hours after stroke onset indicates successful clot lysis <6 hours in MCA/M1 occlusion with a high degree of accuracy. Thus, determination of a single S100B value may serve as a surrogate marker of early and sufficient MCA/M1 recanalisation in large scale thrombolytic studies.

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The primary end point in previous thrombolytic stroke trials was the improvement of functional outcome as measured by various scales such as the modified Rankin scale or the Barthel index.^{1–5} This approach is limited by the low statistical power of these functional scales because of their ordinal scoring level. Additionally, dichotomisation, which is often used to analyse the data with contingency tables, may strongly influence the results derived from these studies.^{6,7} Thus, positive findings concerning functional outcome should be supported by corresponding secondary end points.

The most plausible surrogate marker would be final infarct volume on brain imaging.⁸ Unfortunately, brain imaging requires high technical expenditure and is expensive. Furthermore, the optimal time point and the best imaging modality used for infarct volume measurements remains undefined.⁸ An additional surrogate marker directly reflecting the thrombolytic property of the drug used is the rate and time point of recanalisation of the occluded cerebral artery.^{5,9–11} Vascular monitoring can be performed using various techniques (for example, intra-arterial angiography (IA), computed tomography angiography, or magnetic resonance imaging; transcranial Doppler sonography (TCD) or duplex sonography).^{5,10–15} However, some of these techniques are invasive and use radiography, others require highest technical standards or skilled sonographers for their successful application in hyperacute stroke patients. These shortcomings hamper the widespread application of any of these diagnostic tests in large scale thrombolytic studies. Thus, there is a need for a simple and reliable indicator for the success of clot lysis in thrombolytic stroke trials.

Previous studies have demonstrated significant correlations between serum concentrations of the astroglial Ca²⁺-binding

protein S100B and infarct volume in patients with acute ischaemic stroke.^{16,17} Therefore, we hypothesised that early and sufficient clot lysis in proximal middle cerebral artery occlusion (MCA/M1) may result in smaller lesion volumes that are directly mirrored in the S100B kinetic. In addition, we attempted to identify the best time point for S100B measurements for the purpose of indicating early and sufficient MCA/M1 recanalisation. To answer these questions, we investigated a consecutive sample of patients with acute MCA/M1 occlusion targeted for thrombolysis, and determined the time point of MCA recanalisation, final infarct volume, and S100B serum kinetic.

METHODS

Patients

We prospectively included 23 patients (65.2% male; mean (SD) age, 70.2 (11.0) years) presenting with focal neurological signs in the MCA distribution (43.5% left hemispheric) with a clearly defined time of onset and no evidence of haemorrhage on initial brain imaging. The mean time (SD) between symptom onset and initial brain imaging was 2.2 (1.2) hours and ranged from 55 minutes to five hours. The diagnosis of MCA/M1 occlusion was based on magnetic resonance (MR) angiography in 18, on IA in two, or on TCD in three patients.^{5,11,18,19} A standardised neurological examination was performed shortly after admission and the National Institute

Abbreviations: IA, intra-arterial angiography; TCD, transcranial Doppler sonography; AUC, area under the curve

of Health Stroke Scale (NIHSS) was used to assess clinical impairment. Four patients (17.4%) received intravenous thrombolytic therapy (0.9 mg/kg rt-PA), four patients (17.4%) underwent intra-arterial thrombolytic therapy (1–1.5/10⁶ IU urokinase), and seven additional patients (30.4%) were treated according to an intravenous-intra-arterial bridging concept also using 0.9 mg/kg rt-PA.²⁰ At hospital discharge, the Barthel Index (BI) and modified Rankin Scale (mRS) score were determined as measures of short-term outcome. Patients with an insufficient temporal bone window for TCD recording were prospectively excluded. This study was performed according to local ethical committee standards. All patients or next of kin gave informed consent for study participation and for the determination of the S100B values from the routinely obtained blood samples (see below).

Laboratory investigations

Venous blood samples were drawn immediately after hospital admission, every eight hours within the first three days, and every 12 hours from day 4 to day 6. Blood samples were centrifuged immediately (2703 g, five minutes), serum was separated and stored at –25°C. For measurement of the S100B serum concentrations we used a commercially available monoclonal two site immunoluminometric assay and a fully automatic LIA-mat system (Byk-Sangtec Diagnostica, Dietzenbach, Germany). This test measures the β subunit of protein S100 as defined by three monoclonal antibodies (SMST 12, SMSK 25, and SMSK 28). The detection limit of this kit is 0.02 μ g/l, and the range of protein S100B serum concentrations in 95% of healthy subjects is reported to be <0.12 μ g/l. Intra-assay and interassay variability varied between 2.8% to 6.4% and 2.2% to 10.7%, respectively. All S100B tests were performed “off line” after clinical data collection was finished by a technician who was unaware of patients clinical course.

Vascular monitoring

In the 11 patients treated with intra-arterial thrombolysis, vascular monitoring was performed angiographically. Restoration of MCA blood flow was graded according to the TIMI (Thrombolysis in Myocardial Infarction) flow grade system,²¹ which has also been successfully applied to the cerebral circulation.⁵ Sufficient recanalisation was defined as TIMI 3 flow grade. In these patients IA was followed by TCD monitoring (see below) of the affected MCA to recognise possible deterioration of blood flow after previously documented recanalisation.

In the 12 remaining patients vascular monitoring was based on repetitive TCD examinations using the recently established TIBI flow grade system.¹⁰ TCD recording was performed discontinuously 1, 2, 3, and 24 hours after the initial investigation confirming MCA/M1 occlusion. The ultrasonic probe was fixed with a head tape for temporal insonation. Sufficient recanalisation was suggested if TIBI flow grade was ≥ 3 within the affected M1 segment.

Infarct volumetry

Infarct volume was based on MR imaging (FLAIR; n=12) or computed tomography (CT, n=9) on day 7 after symptom onset. For infarct volumetry on MR images we used a commercially available software (MRVision, MRVision Inc, Winchester, MA, USA), for CT scans we used public domain software from the National Institute of Health, USA.¹⁵ Two patients died between day 4 and day 6 after symptom onset as a result of malignant brain oedema before follow up imaging could be obtained. Infarct volumetry was performed by two neuroradiologists unaware of clinical data (RdMdR, FEZ).

Statistical analysis

Additional to the measured S100B values, the area under the curve (AUC) and the peak value were derived from each indi-

Table 1 Correlations (Pearson's ρ coefficient) between final lesion volume (computed or magnetic resonance tomography at day 7 after stroke onset) and several serum S100B values, the S100B area under the curve (AUC), and the S100B peak value in n=21 patients with proven proximal middle cerebral artery occlusion (MCA/M1). Because of death before day 7, final infarct volume could not be determined in two patients in the group without early recanalisation

S100B measurement	Early (<6 h) MCA/M1 recanalisation	
	Observed (n=7)	Not observed (n=14)
Baseline	0.503	0.089
8 h	0.901	0.457
16 h	0.948*	0.673
24 h	0.976*	0.764*
48 h	0.987*	0.820*
72 h	0.991*	0.847*
96 h	0.988*	0.878*
Peak	0.980*	0.923*
AUC	0.989*	0.893*

*According to the modified Bonferroni correction (α adjustment), we used a p value of <0.0056 (0.05/9=0.0056) to indicate significant findings.

vidual S100B kinetic. S100B measures were compared using the non-parametric Mann-Whitney U test. Pearson's ρ coefficient was applied to verify correlation between S100B measures and infarct volume. Accuracy measures were calculated from cross tabulations (Fisher's exact test) based on cut off points for the above mentioned S100B measures previously derived from receiver operating calculation analyses providing optimised sensitivities and specificities. Because several consecutive statistical tests were performed, α adjustment according to the modified Bonferroni procedure was applied. Additionally, Mann-Whitney U test or Fisher's exact test were used to compare clinical scores and infarct volumes, respectively. All statistical analyses were performed using the SPSS (10.0.7) software package.

RESULTS

Of the 23 patients included in the study seven (30.4%) showed sufficient recanalisation (TIMI=3 or TIBI ≥ 3) of the affected MCA/M1 segment <6 hours after symptom onset (in two patients evidenced by IA, in five patients evidenced by TCD). The median NIHSS score at hospital admission did not differ significantly between the patients with or without early recanalisation (16 (3.6) versus 19 (3.1) points; p=0.115). However, five of seven patients (71.4%) with early recanalisation reached a BI ≥ 70 at hospital discharge, compared with 0 of the 16 remaining patients (p<0.001). Additionally, in the group in which early MCA/M1 recanalisation occurred, two of seven patients (28.6%) reached a mRS score of ≤ 1 at hospital discharge in comparison to 0 of 16 among the others (p=0.025).

Final infarct volume measured at day 7 after stroke onset ranged from 0.0 to 554.5 ml (n=21; mean (SD), 229.3 (180.3) ml). Final lesion volume was 40.6 (61.5) ml versus 317.4 (144.9) ml in the patients with versus without early recanalisation (p<0.001). As shown in table 1, there were significant positive correlations between final lesion volume and each single S100B value obtained between 24 hours and 96 hours after stroke onset, the S100B AUC, and the S100B peak value in both groups, respectively. Furthermore, all S100B measures were lower in the patient group in which sufficient clot lysis of the occluded MCA/M1 segment occurred within six hours (table 2). But, after α adjustment, these differences were only significant for single S100B values obtained 48 hours to 96 hours after stroke onset, for the S100B AUC and for the S100B

Table 2 Mean (SD) values (range) of several serum S100B values, of the S100B area under the curve (AUC), and of the S100B peak value in n=23 patients with confirmed proximal middle cerebral artery occlusion (MCA/M1)

S100B measurement	Early (<6 hrs) MCA/M1 recanalisation		P*
	Observed (n=7)	Not observed (n=16)	
Baseline (µg/l)	0.10 (0.03) (0.05–0.14)	0.15 (0.08) (0.06–0.36)	0.089
8 h (µg/l)	0.11 (0.02) (0.09–0.14)	0.28 (0.38) (0.07–1.53)	0.076
16 h (µg/l)	0.19 (0.18) (0.07–0.58)	1.20 (1.47) (0.07–4.71)	0.039
24 h (µg/l)	0.27 (0.34) (0.08–1.02)	1.61 (1.59) (0.07–5.09)	0.010
48 h (µg/l)	0.37 (0.63) (0.07–1.79)	3.49 (2.12) (0.71–6.84)	<0.001*
72 h (µg/l)	0.30 (0.40) (0.08–1.19)	3.76 (2.43) (0.60–7.23)	<0.001*
96 h (µg/l)	0.24 (0.25) (0.09–0.81)	4.42 (3.18) (0.50–9.46)	<0.001*
AUC (µg/l/h)	22.2 (40.1) (1.3–114.2)	406.8 (284.4) (45.9–911.1)	<0.001*
Peak (µg/l)†	0.58 (0.99) (0.10–2.82)	5.44 (3.61) (0.73–12.20)	<0.001*

*According to the modified Bonferroni correction (α adjustment), we used a p value of <0.0056 (0.05/9=0.0056) to indicate significant findings. †Occurrence of the S100B peak after stroke onset was not significantly different between groups (58.2 (25.7) h for recanalisation observed versus 77.8 (28.7) h for recanalisation not observed, p=0.19, Mann-Whitney U test).

Table 3 Accuracy measures indicating early (<6 h) and sufficient recanalisation of proximal middle cerebral artery occlusion (MCA/M1) for several serum S100B values, for the S100B area under the curve (AUC), and for the S100B peak value in n=23 patients. The corresponding cut off points (CP) were derived from receiver operator characteristic curve analyses to get highest sensitivities and specificities

S100B measurement	Sens	Spec	PPV	NPV	OA	p
Baseline (CP=0.13 µg/l)	0.86	0.69	0.55	0.92	0.74	0.027
8 h (CP=0.15 µg/l)	1.0	0.63	0.54	1.0	0.77	0.007
16 h (CP=0.12 µg/l)	0.71	0.81	0.63	0.87	0.78	0.026
24 h (CP=0.16 µg/l)	0.71	0.93	0.83	0.88	0.87	0.003*
48 h (CP=0.40 µg/l)	0.85	1.0	1.0	0.94	0.96	<0.001*
72 h (CP=0.40 µg/l)	0.85	1.0	1.0	0.94	0.96	<0.001*
96 h (CP=0.40 µg/l)	0.85	1.0	1.0	0.94	0.96	<0.001*
AUC (CP=30 µg/l/h)	0.85	1.0	1.0	0.94	0.96	<0.001*
Peak (CP=0.5 µg/l)	0.85	1.0	1.0	0.94	0.96	<0.001*

*Fisher's exact test (according to the modified Bonferroni correction, we used a p value of <0.0056 (0.05/9=0.0056) to indicate significant findings). Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; OA, overall accuracy.

peak value, respectively (table 2). Using receiver operator characteristic curve analyses, a cut off point of 0.4 µg/l S100B (range 0.27–0.49 µg/l) obtained between 48–96 hours after stroke onset provided a 85.7% sensitivity and 100% specificity indicating sufficient clot lysis within the first six hours (table 3). Before this time interval, a single S100B value did not reliably discriminate between the patient groups. Also shown in table 3, the overall accuracy of a single S100B value obtained in this time window was equally high (95.7%) as it was for the S100B AUC (cut off point 30 µg/l/h) or for the S100B peak value (cut off point 0.5 µg/l).

DISCUSSION

A single S100B value less than 0.4 µg/l obtained 48–96 hours after stroke onset indicates early and sufficient MCA/M1 reperfusion <6 hours after symptom onset with a very high degree of accuracy in hyperacute stroke patients presenting with an MCA/M1 occlusion. Therefore, this simple S100B measurement may provide an easy and reliable surrogate marker for early and sufficient clot lysis in hyperacute thrombotic stroke trials.

The most obvious linkage between MCA recanalisation and S100B kinetic is infarct volume. Previous studies revealed a strong correlation between S100B values (that is, AUC or peak value) and infarct size in consecutive stroke patients.^{16,17} However, both the determination of the S100B peak value or the AUC require multiple consecutive blood analyses. Surprisingly, in this sample of a prospectively defined subset of hyperacute stroke patients, a single S100B value obtained 48–96 hours after stroke onset was at least as predictive as the AUC both for

final infarct volume and early MCA/M1 recanalisation (see tables 1 and 3). Therefore, it can be concluded that the S100B serum kinetic reflects the evolving cerebral infarction that is substantially smaller in cases of sufficient clot lysis in the first six hours. This conclusion was previously suggested by a post hoc analysis of the imaging data of the NINDS rt-PA stroke trial revealing significantly smaller lesion volumes in the rt-PA treated group.⁸ Recently, several authors confirmed this observation. Schellinger *et al* found significantly smaller infarct volumes on T2 weighted MR images in the recanalised group five days after MCA stroke.¹⁵ Molina *et al* reported a mean (SD) infarct volume of 24 (17.8) ml in patients who completely recanalised within six hours in contrast with 131.8 (80.5) ml in those with persistent MCA occlusion at six hours using CT between day 5 and 7.¹¹

The fact that the S100B_{48–96hours} value is merely an indirect indicator of recanalisation may harbour some potential pitfalls. Firstly, false positive findings (S100B_{48–96hours} >0.4 µg/l and sufficient MCA/M1 recanalisation <6 hours) are conceivable if the infarct has already evolved before sufficient restoration of blood flow occurred. This was indeed the case in one of our patients who presented with a large lesion on initial diffusion weighted MR imaging and spontaneous MCA/M1 recanalisation 4.5 hours after stroke onset. Secondly, a false negative case with persisting MCA/M1 occlusion beyond six hours and S100B_{48–96hours} <0.4 µg/l was not observed in our series. Thirdly, the S100B cut off point differentiating between early and late or no recanalisation may be different in stroke patients with more distal vessel occlusions and should be determined in future investigations.

There is convincing evidence that early recanalisation (<6 hours after symptom onset) of the occluded vessel in acute ischaemic stroke is one of the major determinants of substantial clinical improvement and reduction of lesion size.^{11 15 22–24} In a recent work, sufficient clot lysis within six hours after stroke onset emerged as the most potent independent predictor of good outcome and independence at three months with an odds ratio of 23.4 (95% confidence intervals 5.4 to 96).¹¹ Until now, IA has been accepted as the gold standard for vascular monitoring also in the cerebral circulation.^{5 25 26} It permits direct visualisation of the thrombus and an exact grading of flow restoration spontaneously or under thrombolytic therapy.^{5 21} Unfortunately, IA is an invasive procedure with a relevant complication rate, it is not suitable for long term monitoring, and the application especially in the intracranial circulation requires skilled neuroradiologists limiting the use in routine clinical practice or clinical investigations.

Recently, TCD has been proposed as an optimal monitoring tool potentially overcoming the above mentioned drawbacks.¹⁹ In comparison to IA, TCD reached a sensitivity of 87.5% and a specificity of 88.6% (overall accuracy, 88.1%) to detect vessel obstruction in the MCA.¹⁹ Additionally, vessel reopening can also be monitored with an accuracy >90% compared with IA.¹² These capabilities of TCD have recently led to the establishment of a flow grading system indicating increasing degrees of flow restoration in MCA/M1 occlusion (that is, TIBI), which has also been used in our study.¹⁰ Nevertheless, the application of TCD in acute stroke patients requires trained technologists and expert interpreters. Additionally, especially in older patients a sufficient temporal bone window is missing in at least 15% of patients.¹⁹ Again, these shortcomings of TCD limit its use as a reliable and valid test for vascular monitoring in large scale interventional studies. Ongoing thrombolytic stroke trials (DIAS or TRUMBI study) use repetitive MR angiography to determine the MCA recanalisation rate,²⁷ which is exceptionally expensive and limits patient recruitment to highly specialised stroke centres.

In conclusion, a single S100B value obtained between 48 and 96 hours after stroke onset may function as an excellent paraclinical marker to assess the impact of thrombolysis in stroke patients with hyperacute MCA/M1 occlusion. Thus, we would propose to use it as a secondary end point in thrombolytic stroke trials. Future studies will be required to confirm these results in larger patient samples, and more important, to elucidate the suitability of S100B indicating early and sufficient clot lysis also in patients with more distal branch occlusions.

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