Original communication

# Association of biomarker S100B and cerebral oximetry with neurological changes during carotid endarterectomy performed in awake patients

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**Summary:** *Background*: This study attempted to correlate neurological symptoms in awake patients undergoing carotid endarterectomy (CEA) under local anaesthesia (LA) with serum concentration of S100B protein and measurement of cerebral oximetry with near-infrared spectroscopy (NIRS). *Patients and methods*: A total of 64 consecutive CEAs in 60 patients operated under LA during an 18-month period were prospectively evaluated. A cerebral oximeter was used to measure cerebral oxygen saturation ($rSO_2$) before and after cross-clamping along with serum concentration of the S100B protein. Selective shunting was performed when neurological changes occurred, regardless of NIRS. Neurological deterioration occurred (neurological symptoms group) in 7 (10.9 %) operations. In 57 (89.1 %) operations, the patients were neurologically stable (no neurological symptoms group). *Results*: The neurological symptoms that occurred after clamping correlated with an increase in the serum level of S100B ($P$ = .040). The cut-off of 22.5 % of S100B increase was determined to be optimal for identifying patients with neurological symptoms. There was no correlation between $rSO_2$ decline and neurological symptoms ($P$ = .675). Two (3.1 %) perioperative strokes occurred. *Conclusions*: We found a correlation between neurological symptoms and serum S100B protein increase. However, because of the long evaluation time of serum S100B, this monitoring technique cannot be performed during CEA.

**Keywords:** Perioperative stroke prevention, neuromonitoring, carotid stenosis, selective shunting

## Introduction

Carotid endarterectomy (CEA) is widely accepted as the appropriate procedure in patients with severe carotid artery stenosis to reduce subsequent ischaemic stroke. The inherent risk of surgery is perioperative stroke. A major cause of cerebrovascular accidents is hypoperfusion during cross-clamping of the internal carotid artery (ICA). The prompt and reliable recognition of insufficient collateralisation is crucial for a good neurological outcome of patients [1]. General use of an indwelling shunt adds to the complexity of the endarterectomy, and it can injure the artery, leading to thromboemboli [2]. Therefore, proper neuromonitoring needs to identify patients who will benefit from shunt placement [3]. Patients undergoing CEA under local anaesthesia (LA) are monitored clinically, which arguably results in a more appropriate shunt insertion compared with other, less-sensitive methods of neuromonitoring [4].

Several biomarkers have been proposed to predict, diagnose, and monitor brain injury. Among the most studied of these is serum protein S100B [5]. The neuroprotein S100B is the B subunit of a calcium-binding protein found in astroglia and Schwann cells. The role of the S100B protein has not been completely elucidated and characterised yet. At nanomolar levels, S100B stimulates axon outgrowth and enhances neuron survival. However, at micromolar levels, it stimulates the expression of inflammatory cytokines and induces apoptosis [6]. S100B in the peripheral blood is a sensitive marker of both blood-brain barrier (BBB) dysfunction and ischaemic brain damage, even during clinically uneventful CEA [7, 8]. The maximum S100 B levels can be detected as early as 20 minutes after brain injury. However, it has a biological half-life of 30 to 113 minutes and is rapidly excreted by the kidney [9].

However, in the past decade, concern has been raised with regard to subtle, subclinical brain damage during

carotid revascularization [10]. Cappocia [11] analysed S100B values as continuous and categorical data and found that to some degree, they can be considered related to subclinical brain injuries. Sahlein [12] inserted a 6-French Fogarty catheter into the facial vein and threaded it 6 cm rostrally into the jugular bulb during CEA. Fifteen minutes after clamping the carotid artery, S100B levels in the jugular bulb were significantly elevated compared with the levels at the baseline. Some reports have described that subtle cerebral injuries after CEA were associated with significant increases in serum S100B levels [13, 14]. Some studies noticed a significant increase in S100B levels after declamping [15, 16], with a tendency to decline 24 hours after surgery [10, 17]. Falkensammer [18] examined subclinical alterations of cerebral function during CEA and the predictability of minor cerebral damage by perioperative levels of the biochemical marker S100B. Aleksic [4] showed low but significant increases in the arterial S100B level during carotid cross-clamping.

Near-infrared spectroscopy (NIRS) is the regional oxygen saturation ($rSO_2$) measurement. In our department, we applied NIRS monitoring during CEA in all patients. NIRS is accomplished by placing a near infrared (NIR) emitting element on the skin over the forehead. Human tissue is translucent to NIR, so a portion of the light passes through the skin, subcutaneous tissues, and bone. The portion of the light that penetrates to the underlying brain is reflected by the cerebral parenchyma. The reflective property of the cerebral tissue largely depends on the oxygenated hemoglobin concentration of the tissue at specific wavelengths. Reflected photons are then detected by the device and the quantity of reflected photons as a function of wavelength is recorded. Tissue oxygenation is inferred by the INVOS system by measuring the quantity of reflected light with 730 and 810 nm wavelengths to determine the regional hemoglobin oxygen saturation ($rSO_2$ index referred elsewhere as simply $rSO_2$) [19].

The aim of this preliminary study was to investigate whether increased serum S100B levels or a drop in $rSO_2$ during the carotid revascularisation by CEA could be used to detect neurological instability in patients undergoing a CEA. We hypothesised that increased serum S100B levels during the CEA would correlate with neurological symptoms during the surgical procedure.

# Patients and methods

## Study population

A prospective observational study design was performed with patients admitted to the Department of Vascular Surgery of Novo Mesto General Hospital who underwent CEA between October 2012 and September 2013. Sixty adults (41 men, 19 women) between the ages of 50 and 86 years who underwent 64 CEAs over a 12-month time period were studied. The approval of the National Medical Ethics Committee of the Republic of Slovenia was obtained; written informed consent was obtained from all the patients.

Indications for CEA included ipsilateral neurological symptoms (stroke, transient ischaemic attack [TIA], amaurosis fugax), with $\geq$ 50 % ICA stenosis, and both symptomatic and asymptomatic patients with 70 % to 99 % stenosis. All the CEAs in the symptomatic patients were performed < 7 days of symptom onset. The CEAs in the asymptomatic patients were performed < 1 month after the diagnosis of carotid stenosis.

## Carotid endarterectomy

Twenty-six patients were symptomatic, and all were scheduled to undergo CEA with regional anaesthesia achieved by the combination of superficial and deep cervical plexus block (100 mg levobupivacaine + 200 mg lidocaine).

After carotid clamping, neurological assessment was performed by having the patient squeeze into the contralateral hand and speak. Neurological assessment was continuous throughout the operative procedure at 3-min intervals. The patients were assigned to one of two groups: those who developed neurological symptoms (neurological symptoms group) during clamping and those who did not (no neurological symptoms group).

Criteria for the neurological symptoms group were development of motor weakness, slurring of speech, inability to respond appropriately to verbal commands, loss of consciousness, or seizure. These were also used as criteria for insertion of a shunt or anticipating a very short clamp time with primary closure.

The patch closure was performed in 58 CEAs, with primary closure in 6. Primary closure was used in four cases where the arteriotomy was short, confined within the bulb, and the surgeon's assessment of an internal carotid artery diameter was > 5 mm. In the two remaining patients who were restless, the surgeon anticipated a very short clamp time and elected to complete the operation with primary closure.

## Cerebral oximetry

The cerebral oximeter INVOS 5100C (Somanetics) was used to measure simultaneous, bilateral $rSO_2$ throughout the procedure.

During surgery, brief and variable degrees of cerebral ischaemia occur during cross-clamping of the ICA [20, 21]. The pre-clamping bilateral $rSO_2$ value and the lowest ipsilateral measurement after ICA clamp placement were recorded.

Intersubject variability in $rSO_2$ index values is well known and was noticed in this study. To facilitate comparison of $rSO_2$ changes after carotid cross-clamp among all patients, and to determine the magnitude of $rSO_2$ change that was associated with a change in neurological function,

 

the $rSO_2$ data were normalised by calculating a percentage change in $rSO_2$ reading during cross-clamp periods in each patient according to a formula: [Percentage change = (mean $rSO_2$ reading preclamp − minimum $rSO_2$ reading crossclamp)/(mean $rSO_2$ reading preclamp)]. On the basis of previous studies [22], a decrease in $rSO_2$ of $\geq$ 12 % was considered clinically significant.

We were monitoring the $rSO_2$ in the contralateral hemisphere as well. With hypotension, falls in ipsilateral and contralateral $rSO_2$ concurrently occurred. These measurements were therefore excluded from the analysis. Bilateral monitoring of $rSO_2$ can aid in distinguishing carotid occlusion from cerebral hypoxemia due to systemic causes.

### Serum biomarker of brain injury

Venous blood samples were obtained for each patient preoperatively (basal sample, preclamp), immediately after the end of the procedure (declamp), and 12 hours, 24 hours, and 48 hours after the surgery. Samples were allowed to clot. Blood samples were centrifuged within 30 minutes, and serum was stored at −20 °C until assayed in duplicate in a single batch within 6 months. The concentrations of protein S100B were measured by automated electrochemiluminescence assay (Cobas e411 analyser, Roche Diagnostics, Mannheim, Germany). The lower limit of detection for protein S100B was 0.005 μg/L. The upper reference limit of protein S100B was set at 0.105 μg/L, representing the 95th percentile of the healthy population. The reference limit was provided by the manufacturer of the assay and verified by the laboratory. We compared the baseline value with the sample taken immediately after the end of the procedure. An increase of 25 % was considered abnormal [11].

### Statistical analysis

The lowest values of $rSO_2$ and highest values of S100B were used for comparison between the no neurological symptoms and neurological symptoms groups. The baseline characteristics of patients who developed neurological symptoms were compared with the patients with no symptoms using the chi-square test or Fisher exact test, where appropriate. In the case of continuous variables the independent samples t-test or Mann-Whitney test was used. Of these results positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR−) and diagnostic odds ratio (DOR) with 95 % confidence intervals (CI) were calculated. ROC curve analysis was applied to identify the threshold values of parameters and the area under the curve (AUC) values were compared. Statistical analysis was performed with R 3.5.2 statistical software (R Foundation for Statistical Computing, Vienna, Austria). A p-value less than 0.05 was considered as statistically significant.

## Results

### Baseline characteristics

In this study, 64 CEAs were performed under regional anaesthesia. There were 41 (67 %) men and 19 (33 %) women; patient mean age (±SD) was 70.9 ± 8.5 years (range, 50–86 years). Two men and two women were operated bilaterally. Fifty-nine per cent of the CEAs were performed for asymptomatic disease, in comparison with 41 % for symptomatic disease.

### Demographics, anatomy, and pre-operative variables

There were no differences (Table I) in diabetes mellitus, hypertension, smoking, hyperholesterolaemia, prior coronary artery bypass graft, and chronic renal failure or peripheral artery disease between those with and without neurological symptoms. There were no significant differences in rates of overall prior carotid intervention, symptomatic features, contralateral carotid stenosis grade, or pre-operative diagnostics between the two groups. However, ipsilateral carotid stenosis grade of 50–69 % (43 % vs. 12 %) were more common in those with neurological symptoms and ipsilateral carotid stenosis grade of 70–89 % (56 % vs. 14 %) were more common in those without neurological symptoms ($P$ = .056).

### Operative and post-operative variables

Neurological deterioration after carotid clamping occurred (neurological symptoms group) in 7 (10.9 %) operations. After insertion of a shunt the neurological changes subsided in all patients. The main symptoms were slurring of speech and loss of consciousness.

The median [1stQ, 3rdQ] duration of carotid cross-clamping was 23.2 [19.8, 28.9] minutes and 20.9 [12.4, 29.2] minutes in the no neurological and neurological symptoms groups, respectively (Table II). This difference was not statistically significant ($P$ = .519). A prosthetic patch was used in 58 (90.6 %) procedures and primary closure in 6 (9.4 %) procedures.

### S100B

Median [1stQ, 3rdQ] baseline serum levels of protein S100B in the asymptomatic patients and symptomatic patients were 0.030 μg/L [0.021 μg/L, 0.053 μg/L] and 0.037 μg/L [0.025 μg/L, 0.060 μg/L], respectively. There were no significant differences in baseline preclamp concentrations of protein S100B between the patients ($P$ = .456; Mann-Whitney U test). No statistically significant difference was observed in any of the categories when comparing the asymptomatic and symptomatic patients (Table III). No statistically significant difference was

**Table I.** Baseline characteristics of no neurological symptoms (NS–) and neurological symptoms (NS+) group

| Baseline characteristics | All patients (n = 64) | NS– (n = 57) | NS+ (n = 7) | p-value |
|---|---|---|---|---|
| Age | 70.9 ± 8.5 | 71.0 ± 8.1 | 70.1 ± 12.1 | 0.811 |
| Gender – male | 43 (67) | 39 (68) | 4 (57) | 0.675 |
| Smoking – current or past | 23 (36) | 20 (35) | 3 (43) | 0.695 |
| Hypertension | 58 (91) | 51 (90) | 7 (100) | 1.000 |
| Diabetes | 30 (47) | 27 (47) | 3 (43) | 1.000 |
| Hypercholesterolaemia | 51 (80) | 46 (81) | 5 (71) | 0.623 |
| Prior PCI | 10 (16) | 10 (16) | – | – |
| Prior CABG | 10 (16) | 9 (16) | 1 (14) | 1,000 |
| PAD | 18 (28) | 17 (30) | 1 (14) | 0.662 |
| CRF | 18(28) | 16 (28) | 2 (29) | 1.000 |
| ***Symptomatic features*** | | | | |
| Symptomatic stenosis | 26 (41) | 22 (39) | 4 (57) | 0.428 |
| TIA | 12 (19) | 10 (18) | 2 (29) | 0.607 |
| CVI | 14 (22) | 12 (21) | 2 (29) | 0.642 |
| ***Prior ipsilateral CEA/CAS*** | | | | |
| Prior contralateral CEA | 6 (9) | 5 (9) | 1 (14) | 0.516 |
| Prior contralateral CAS | 1 (2) | 1 (2) | – | – |
| ***Ipsilateral carotid stenosis grade*** | | | | |
| 50–69% | 10 (16) | 7 (12) | 3 (43) | |
| 70–89% | 33 (51) | 32 (56) | 1 (14) | 0.056 |
| ≥90% | 21 (33) | 18 (32) | 3 (43) | |
| ***Contralateral carotid stenosis grade*** | | | | |
| <50% | 34 (61) | 32 (64) | 2 (33) | |
| 50–69% | 12 (21) | 9 (18) | 3 (50) | 0.215 |
| ≥70% | 10 (18) | 9 (18) | 1 (17) | |
| Contralateral carotid occlusion | 8 (12) | 7 (12) | 1 (14) | |
| ***Pre-operative diagnostics*** | | | | |
| Pre-operative duplex ultrasound | 46 (72) | 41 (72) | 5 (71) | 1.000 |
| Pre-operative CTA | 52 (81) | 46 (81) | 6 (86) | 1.000 |

Results are presented as n (%) or mean ± standard deviation. PCI = percutaneous coronary intervention; CABG = coronary artery bypass graft; PAD = peripheral arterial disease; CRF = chronic renal failure; TIA = transient ischaemic attack; CVI = cerebrovascular insult; CEA = carotid endarterectomy; CAS = carotid artery stenting; CTA = computer tomographic angiography.

**Table II.** Operative in post-operative factors in patients without neurological symptoms (NS–) and patients with neurological symptoms (NS+) during CEA.

| Factor | NS– (n = 57) | NS+ (n = 7) | p-value |
|---|---|---|---|
| Length of cross-clamping (min.) | 23.2 [19.8–28.9] | 20.9 [12.4–29.2] | 0.519 |
| **$rSO_2$** | | | |
| Base ipsilateral | 69 [62–75] | 62 [54–72] | 0.232 |
| Lowest ipsilateral | 60 [52–67] | 52 [42–65] | 0.297 |
| Post-clamp ipsilateral (% decrease) | 10 [6–19] | 15 [6–21] | 0.675 |
| Base contralateral | 69 [63–75] | 65 [48–73] | 0.232 |
| Lowest contralateral | 65 [60–72] | 51 [41–64] | 0.042 |
| Post-clamp contralateral (% decrease) | 4 [2–8] | 4 [2–15] | 0.731 |
| **S100B** | | | |
| Pre-clamp (μg/L) | 0.03 [0.02–0.05] | 0.05 [0.02–0.07] | 0.771 |
| Declamp (% increase) | –3 [–31–25] | 36 [7–79] | 0.040 |
| Post op 12 h (% increase) | –28 [–46 to –2] | 9 [–35–26] | 0.141 |
| Post op 24 h (% increase) | –17 [–39–14] | 4 [–26–32] | 0.432 |
| Post op 48 h (% increase) | –8 [–33–53] | 11 [–45–51] | 0.755 |

Results are presented as median [interquartile range]. CEA: carotid endarterectomy; $rSO_2$: cerebral oxygen saturation; S100B: protein S100B.

 

**Table III.** Operative in post-operative factors in symptomatic (S) and asymptomatic (A) patients.

| Factor | S (n = 26) | A (n = 38) | p-value |
|---|---|---|---|
| Length of cross-clamping (min.) | 22.5 [19.2–29.6] | 23.2 [20.3–28.2] | 0.677 |
| **$rSO_2$** | | | |
| Base ipsilateral | 69 [64–75] | 68 [61–72] | 0.452 |
| Lowest ipsilateral | 61 [53–66] | 58 [51–66] | 0.599 |
| Post-clamp ipsilateral (% decrease) | 11 [6–23] | 10 [6–17] | 0.613 |
| Base contralateral | 68 [65–72] | 70 [60–76] | 0.538 |
| Lowest contralateral | 65 [61–68] | 64 [57–73] | 0.763 |
| Post-clamp contralateral (% decrease) | 3 [2–7] | 5 [2–11] | 0.507 |
| **S100B** | | | |
| Pre-clamp (μg/L) | 0.04 [0.03–0.06] | 0.03 [0.02–0.05] | 0.460 |
| Declamp (% increase) | 3 [−27–44] | −2 [−28–28] | 0.618 |
| Post op 12 h (% increase) | −28 [−40 to −7] | −26 [−46–5] | 0.908 |
| Post op 24 h (% increase) | −12 [−37–16] | −17 [−37–21] | 0.941 |
| Post op 48 h (% increase) | −9 [−38–36] | −5 [−26–55] | 0.484 |

Results are presented as median [interquartile range]. CEA: carotid endarterectomy; $rSO_2$: cerebral oxygen saturation; S100B: protein S100B.

**Table IV.** Operative in post-operative factors in symptomatic (S-TIA or S-CVI) and asymptomatic patients (A).

| Factor | S-TIA (n = 12) | S-CVI (n = 14) | A (n = 38) | p-value |
|---|---|---|---|---|
| Length of cross-clamping (min.) | 19.3 [19.1–26.0] | 25.0 [21.3–30.2] | 23.2 [20.3–28.2] | 0.339 |
| **$rSO_2$** | | | | |
| Base ipsilateral | 70 [65–76] | 69 [59–72] | 68 [61–72] | 0.622 |
| Lowest ipsilateral | 61 [56–64] | 61 [50–68] | 58 [51–66] | 0.855 |
| Post-clamp ipsilateral (% decrease) | 11 [7–22] | 11 [6–22] | 10 [6–17] | 0.872 |
| Base contralateral | 70 [66–71] | 67 [61–72] | 70 [60–76] | 0.687 |
| Lowest contralateral | 67 [63–68] | 64 [54–66] | 64 [57–73] | 0.451 |
| Post-clamp contralateral (% decrease) | 3 [2–3] | 5 [2–7] | 5 [2–11] | 0.423 |
| **S100B** | | | | |
| Pre-clamp (μg/L) | 0.03 [0.02–0.05] | 0.04 [0.03–0.06] | 0.03 [0.02–0.05] | 0.500 |
| Declamp (% increase) | 21 [−28–53] | −3 [−26–19] | −2 [−28–28] | 0.701 |
| Post op 12 h (% increase) | −27 [−42–0.1] | −30 [−38 to −19] | −26 [−46–5] | 0.971 |
| Post op 24 h (% increase) | 0.1 [−20–37] | −25 [−38 to −2] | −17 [−37–21] | 0.374 |
| Post op 48 h (% increase) | −0.5 [−12–12] | −29 [−46–49] | −5 [−26–55] | 0.660 |

Results are presented as median [interquartile range]. CEA: carotid endarterectomy; $rSO_2$: cerebral oxygen saturation; S100B: protein S100B.

observed in any of the categories when comparing the asymptomatic patients, post-CVI patients, and post-TIA patients (Table IV).

Percentage of increase in S100B parameter at different timeframes for the no neurological symptoms and neurological symptoms groups are depicted in Figure 1.

The median serum S100B level increase was 36.2 % [6.9 %, 79.0 %] (median [1stQ, 3rdQ]) in the neurological symptoms group, compared with −2.7 % [−30.9 %, 25.5 %] (median [1stQ, 3rdQ]) in the no neurological symptoms group. The highest increase of serum S100B protein was 209 %. The increase was significantly different between the groups ($P = .040$; Mann-Whitney U test) (Figure 1). This finding indicates that neurological instability that occurs after clamping correlates with the increase in S100B level.

Neurological change could be predicted as a function of a 25 % increase of S100B marker. Applying this technique, the area under the curve (AUC) was 0.7393 (95 % confidence interval CI = [0.5472, 0.9315]), and the diagnostic sensitivity and specificity were 71.4 % and 75.4 %, respectively. The threshold for S100B of 22.5 % increase was optimal to identify patients with neurological symptoms (Figure 2, Table V).

## Cerebral oxygen saturation

The decrease in $rSO_2$ from the preclamp to cross-clamp period on the ipsilateral side was not statistically significant between the groups. The median $rSO_2$ decrease was 15 % [6 %, 21 %] (median [1stQ, 3rdQ]) in the neurological symptoms group vs. 10 % [6 %, 19 %] (median [1stQ, 3rdQ]) in the no neurological symptoms group ($P = .675$, Mann-Whitney U-test).

The correlation between changes in $rSO_2$ and neurological symptoms was analysed and by ROC analysis, a cut-off of $rSO_2$ decrease of 13.4 % was determined to be optimal

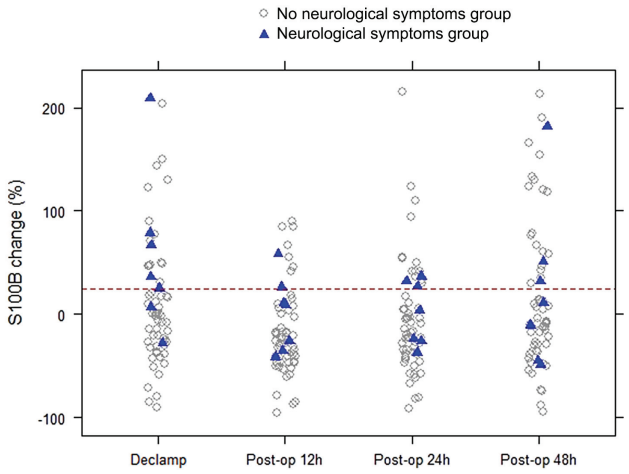


**Figure 1.** Percentage of increase in S100B parameter at different timeframes for the no neurological symptoms and neurological symptoms groups.

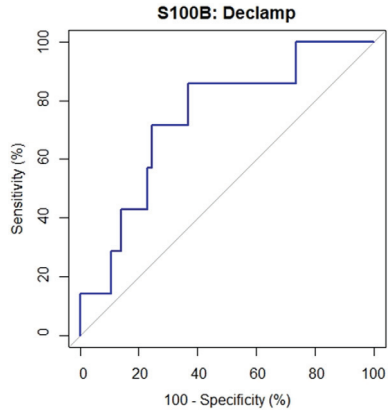


**Figure 2.** ROC curve for performance of S100B declamp percentage change in prediction of neurological symptoms. The closest top left point is at threshold value 22.5 % with sensitivity of 71.4 % and specificity of 75.4 %. The area under the curve is 74 % (95 % CI: 55–93 %).

for identifying patients with neurological symptoms, AUC = 0.5489, 95 % CI = [0.3327, 0.7651]. Sensitivity and specificity were 57.1 % and 63.2 %, respectively (Figure 3, Table V).

## Contralateral carotid occlusion

There were no statistically significant differences between contralateral carotid occlusion and neurological symptoms ($P$ = 1), contralateral carotid occlusion and serum S100B increase ($P$ = .466), or contralateral carotid occlusion and $rSO_2$ fall ($P$ = .418).

## Prediction of neurological symptoms

The PPV for the prediction of neurological symptoms during the CEA was 16 % for the $rSO_2$ parameter; for the S100B parameter the values were 26 % for declamp, 22 % for 12 h post-operative, 19 % for 24 h post-operative, and 14 % for 48 h post-operative. The NPV for the $rSO_2$ parameter and S100B parameter at different timeframes were 92 %, 96 %, 91 %, 92 % and 91 %, respectively (Table VI). However, in terms of LR considered as minimally predictive, the declamp S100B parameter had amongst other measurements the highest LR+ (2.9, 95 % CI: 1.5–5.6) and lowest LR– (0.4, 95 % CI: 0.1–1.2). Also in terms of DOR, this parameter performed best (7.7, 95 % CI: 1.4–44.0).

## Peri-operative outcomes

Two (3.1 %) perioperative strokes occurred. The single major stroke happened on day 6, and the patient had decreased responsiveness to verbal commands and developed left hemiplegia. The diagnosis of stroke was based on the clinical and computed tomography finding of a focal ipsilateral ischaemic cerebral infarct with a patent CEA site on angiography. The patient was asymptomatic. The other patient had mild neurological deficits with complete recovery without re-exploration of the carotid artery. The patient was symptomatic. Both patients were shunted, and had normal $rSO_2$, and increased S100B markers.

# Discussion

The main finding of this pilot study was that an increase in serum S100B protein level as a marker of cerebral injury correlates significantly with neurological instability. Statistically significant difference was solely detected from the declamp measurement, and were missed based on the 12 h, 24 h and 48 h post-operative measurements. An early mechanism of S100B release occurring within minutes of an inciting event is as yet undefined. One possible cause for early elevations in serum S100B may be that low-level hypoperfusion associated with carotid cross-clamping results in mild blood-brain barrier dysfunction [12, 23]. Although S100B is associated with cerebral ischaemia, successful translation into a biomarker useful in clinical practice for differential diagnosis has proven difficult [24, 25]. Many authors have stated that elevated levels of S100B in the blood are not specific for stroke, as increases occur in other neurological conditions that are symptomatically similar [26].

Seven (10.9 %) patients who required shunting developed cerebral ischaemia (neurologic deficit) during carotid clamping. These results are similar to those reported by Hans [27] (10 %), Evans [28] (9.7 %), Calligaro [29] (7.2 %), Stroughton [30] (14 %), and Rockman [31] (11 %) for CEA in awake patients. In particular, of the 7 patients who required shunting due to the lack of compensatory blood flow, five patients were in the S100B-positive group.

We found that neurologic change could be predicted as a function of S100B marker increase with a sensitivity and specificity of 71.4 % and 75.4 %, respectively. The cut-off value for neurological symptoms was an increase of 22.5 %. Measurements of serum S100B protein for

 

**Table V.** Results of ROC curve analysis with threshold values.

| | AUC (%) | Cut-off (%) | Sensitivity (%) | Specificity (%) | Accuracy (%) |
|---|---|---|---|---|---|
| **$rSO_2$** | | | | | |
| Post-clamp ipsilateral | 55 (33–77) | 13.4 | 57 (14–86) | 63 (51–75) | 63 (22–83) |
| Post-clamp contralateral | 46 (18–74) | −2.2 | 14 (0–43) | 98 (93–100) | 89 (22–83) |
| **S100B** | | | | | |
| Declamp | 74 (55–93) | 22.5 | 71 (29–100) | 75 (65–86) | 66 (58–88) |
| Post-operative, 12 h | 67 (47–88) | 8.4 | 57 (14–86) | 83 (72–91) | 80 (31–89) |
| Post-operative, 24 h | 59 (40–78) | −37.6 | 100 | 28 (16–40) | 36 (30–83) |
| Post-operative, 48 h | 54 (29–78) | 10.4 | 57 (29–86) | 61 (49–74) | 61 (19–92) |

Results are presented as value (95% CI). AUC: area under the curve; $rSO_2$: cerebral oxygen saturation; S100B: protein S100B.

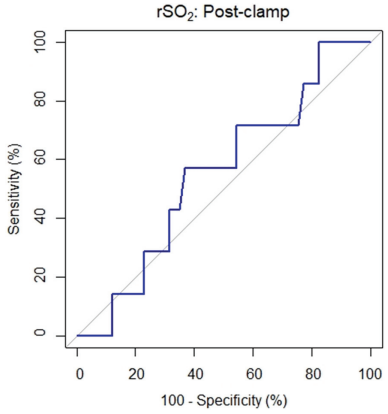


**Figure 3.** ROC curve for performance of $rSO_2$ post-clamp percentage change in prediction of neurological symptoms. The area under the curve is 55 % (95 % CI: 33–77 %).

perioperative neuromonitoring cannot currently be performed because of the long assay procedure. Existing assays lack sensitivity and ease of use and usually require $\geq$ 3 h to perform. We would need a rapid and sensitive immunoassay for determination of S100B protein. Moreover, the observed increase in serum S100B protein during cross-clamping under LA was less pronounced compared with data obtained from carotid surgery under general anaesthesia (GA). The mean increase of S100B in our study was 12 %. Aleksic [4] reported a similar increase in serum S100B levels during CEA in LA, 18 %. Jaranyi [32] reported a 170 % increase in serum S100B levels during CEA in patients operated on under GA. This indicates the relevant global cerebral ischaemia. The S100B levels and associated cerebral ischaemic changes in patients who underwent CEA under LA probably do not apply to patients undergoing CEA under GA [29].

We found higher preclamp S100B concentrations in symptomatic patients than in asymptomatic patients. The median baseline serum levels of protein S100B values were 0.037 µg/L and 0.030 µg/L, respectively. The difference was not statistically significant ($P$ = .456). Maximum levels of S100B can be detected as early as 20 minutes after brain injury. However, it has a biological half-life of 30 to 113 minutes, and is rapidly excreted by the kidney. The concentrations return to near pre-clamp values 24 hours after surgery and subsequent restore by the third postoperative day. All the symptomatic patients in our study were operated later than 72 hours after the carotid territory symptoms. No significant differences in baseline preclamp concentrations of protein S100B between the asymptomatic and symptomatic patients were expected. Dragas [10] found significantly higher preclamp S100B concentrations in symptomatic patients. This was explained by the greater embolic potential of symptomatic carotid plaques.

The decrease in $rSO_2$ from the preclamp to cross-clamp period on the ipsilateral side was not significantly greater in the neurological symptoms group ($P$ = .675). Moreover, the low AUC value suggests that the correlation between percentage drop in $rSO_2$ and neurological symptoms is a weak one at best. Relative drop in $rSO_2$ is neither sensitive (57.1 %) nor specific (63.2 %) in detecting patients with neurological symptoms. These data do not support the

**Table VI.** Performance of $rSO_2$ and S100B parameters in prediction of neurological symptoms.

| | PPV (%) | NPV (%) | LR+ | LR− | DOR |
|---|---|---|---|---|---|
| $rSO_2$ | 16 (8–28) | 92 (83–97) | 1.6 (0.8–3.2) | 0.7 (0.3–1.6) | 2.3 (0.5–11.2) |
| S100B | | | | | |
| Declamp | 26 (16–41) | 96 (87–99) | 2.9 (1.5–5.6) | 0.4 (0.1–1.2) | 7.7 (1.4–44.0) |
| Post-operative, 12 h | 22 (7–53) | 91 (86–94) | 2.3 (0.6–9.1) | 0.8 (0.5–1.3) | 2.9 (0.5–17.6) |
| Post-operative, 24 h | 19 (8–38) | 92 (85–96) | 1.9 (0.7–5.0) | 0.7 (0.4–1.4) | 2.5 (0.5–12.8) |
| Post-operative, 48 h | 14 (6–30) | 91 (83–95) | 1.4 (0.5–3.5) | 0.8 (0.4–1.6) | 1.6 (0.3–8.0) |

Results are presented as value (95% CI). DOR: odds ratio; LR−: negative likelikood ratio; LR+: positive likelikood ratio; NPV: negativne predictive value; PPV: positive predictive value; protein S100B; $rSO_2$: cerebral oxygen saturation; S100B.

use of cerebral oximetry as the sole monitoring modality during carotid endarterectomy.

The absolute lowest value of $rSO_2$ on the contralateral side was statistically different ($P$ = .042) comparing the groups. This has no clinical implication, since there is considerable inter-individual variation in baseline measures of $rSO_2$ [33, 34]. There is a wide range of baseline $rSO_2$ values, varying from 47 % to 86 %. It is appropriate to use relative changes in $rSO_2$, rather than absolute values [35].

One limitation of currently available $rSO_2$ monitoring technology is that the oxygen sensor can be applied to only the hair-free area of the scalp. Focal cerebral ischaemia in other parts of the brain may develop without a decrease in $rSO_2$ registered by the sensors placed on the forehead [20].

Secondary dilatation of the microcirculation due to autoregulation or opening of collateral vessels occurs after an abrupt drop in the blood flow in the brain tissue (which might include an exponential decline in the tissue oxygenation) with the correction for drifting blood pressure [36]. Though intracranial collateral anastomoses may be present, they are insufficient to maintain intracerebral oxygen saturation at the initial levels. Moderate falls in $rSO_2$ were not associated with cerebral hypoperfusion of sufficient severity to cause cerebral ischaemia. The critical fall of $rSO_2$ that the brain can tolerate is not known yet [1].

In our study, the 13 % (13.4 %) fall in $rSO_2$ was identified as optimal to identify patients with neurological symptoms with a sensitivity and specificity of 57.1 % and 63.2 %, respectively. The result was similar to Al-Rawi [21] and Mille [22] with cut-off value of 13 % and 12 % (11.7 %), respectively.

There were 8 patients with the presence of a contralateral carotid occlusion. One fell in the neurological symptoms group, one had an increased serum level of S100B, and two had a significant fall of $rSO_2$. Contralateral carotid occlusion does not reliably predict the need for a shunt.

Two patients suffered cerebrovascular insult (CVI). The patient who suffered CVI during the procedure had the highest increase of serum S100B protein among all patients, but her $rSO_2$ was normal. More extensive cerebral injuries are associated with higher serum S100B levels with relatively late peak times. Patients with subclinical cerebral tissue death exhibit lower and progressively earlier peak serum levels [37]. The other patient suffered a CVI on day 6 after the procedure. The increase in serum S100B protein was significant, and the $rSO_2$ remained normal. The neurological instability and increased level of S100B predicted the CVI attack. This was not true for $rSO_2$.

## Limitations

This study had several limitations. First, the study was limited due to the relatively small number of patients with neurological symptoms during the procedure, although the overall patient population was quite large. Second, the difficulty in the use of S100B biomarker to diagnose brain ischemia resulted in the reported elevation of S100B levels in the blood of hypertensive patients compared to healthy controls [38]. We didn't compare the hypertensive and non-hypertensive subgroups regarding serum S100B elevation. Third, the evaluation of neurological symptoms during the operative procedure may vary from a surgeon to a surgeon.

## Conclusions

Awake neuromonitoring is inherently specific for CEA under LA and has been shown to be a sensitive direct measure of cerebral tissue perfusion. Although positive association was identified between neurological symptoms during the CEA and serum S100B protein increase, the monitoring of serum S100B during the CEA cannot be performed because of the long evaluation time (it usually requires $\geq$ 3 h to perform). Firm conclusions cannot be drawn due to a small number of patients involved in the study, especially in the neurological symptoms group. Future studies will confirm our results or will confute them.

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