# S100β and Nitric Oxide Product Concentrations Following Cerebral Aneurysm Clipping in Patients With Subarachnoid Haemorrhage: A Pilot Study

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**ABSTRACT**

**Objective:** *The objective of this investigation was to determine the efficacy of S100β and nitric oxide product (nitrate and nitrite [NOx]) concentrations as markers of brain injury following cerebral aneurysm clipping in patients with spontaneous subarachnoid haemorrhage.*

**Methods:** *Fifteen patients with spontaneous subarachnoid haemorrhage were studied. Blood samples were obtained for estimation of serum S100β (μg/L) and nitric oxide product (nitrate and nitrite [NOx]) (μM) concentrations immediately preoperatively (baseline) and then 10 minutes, 2, 6 and 12 hr postoperatively and daily thereafter for five days. Neurological outcome was assessed three months after surgery by the Glasgow Outcome Scale (GOS) (poor outcome, grade 1 - 3 and good outcome as grade 4 - 5). Data were analysed using the Mann -Whitney-U-test.*

**Results:** *S100β concentrations were greater at two hours postoperatively compared to baseline (0.33 ± 0.16 vs 0.25 ± 0.04)(P = 0.02). S100β concentrations were similar in good and poor neurological outcome groups as defined by GOS at three months. NOx concentrations were less at 12 hours postoperatively compared to baseline (9.81 ± 3.25 vs 12.74 ± 2.9)(P = 0.03). NOx concentrations were greater on the fourth and fifth postoperative days compared to baseline (t0) (17.22 ± 7.9, 12.74 ± 2.9 vs 9.81 ± 3.25) (P < 0.05). NOx concentrations were greater in patients with a poor neurological outcome (n = 4) compared to the good outcome group (n = 11) (24.7 ± 4.9 vs. 11.3 ± 3.3)(P = 0.04).*

**Conclusions:** *S100β and NOx concentrations increase after cerebral aneurysm clipping in patients with spontaneous SAH. Increased nitric oxide product concentrations were associated with subsequent poor neurological outcome.* (**Critical Care and Resuscitation 2005; 7: 292-297**)

**Key words:** Brain injury, nitric oxide, S100β, subarachnoid haemorrhage

Despite advances in surgical techniques and pharmacological treatment, aneurysmal subarachniod haemorrhage (SAH) still results in profound neurological deficits in many patients.[1] Primary and secondary brain injury is difficult to quantify. At present standard methods of assessing brain injury are the Glasgow Coma Scale (GCS) and computed tomography (CT). Both of these have important limitations.

S100β protein has been extensively studied as marker of neurological injury. It is a dimeric, acidic, calcium binding protein which exists in various forms depending on its α or β chain structure. The ββ form (S100 β) is found in astroglial and Schwann cells.[2] It is also present in lower concentrations in adipose tissue

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and chondrocytes.[2] Positive correlations have been demonstrated between significantly increased concentrations of S100β and worse neurological outcome in patients with head injury,[3,4] stroke,[5,6] subarachnoid haemorrhage,[7-9] and following coronary artery bypass graft surgery.[10,11] Characterisation of S100β release after cerebral aneurysm clipping in patients with spontaneous SAH has not been reported. The increase in plasma S100β concentration following surgery may reflect the effect of surgical intervention.

Nitric oxide (NO) has well described physiological properties including the regulation of vascular smooth muscles.[12] It is also present in neurons, and in other components of the immune system.[12,13] Nitric oxide production is greatly increased in the acutely ischaemic brain and plays a role in the modulation of neurotransmission, synaptic plasticity and cerebral vasomotor control.[14] After head injury in humans, increased cerebrospinal fluid (CSF) and plasma nitrite and nitrate concentrations are associated with worse injury severity scores.[15]

Therefore, biochemical markers of neurological injury may be used in quantification of brain injury severity. Clinically this may facilitate early therapeutic interventions and prediction of the neurological outcome. In order to quantify the effect of surgery in this investigation S100β and NO concentrations following cerebral aneurysm clipping in patients with SAH were assessed. Association with subsequent neurological outcome was also studied.

## MATERIALS and METHODS

With institutional ethical approval and having obtained written informed consent from a first-degree relative, 15 patients with spontaneous SAH (documented either by CT or lumbar puncture) were studied. Patients with a history of cardiac or pulmonary disease, organic brain disease, substance abuse, renal or hepatic failure, body mass index > 32 and pregnant patients were excluded. After initial stabilisation, the clinical severity of SAH was classified using the Hunt and Hess Scale.[16]

With standard monitoring in place, including direct arterial pressure monitoring, general anaesthesia was induced with intravenous propofol (1 - 2 mg/kg), fentanyl (2 - 3 μg/kg) and muscle relaxation was achieved with vecuronium (0.1 - 0.2 mg/kg). Anaesthesia was maintained with isoflurane in oxygen (40%) and nitrous oxide (60%). Post-operatively patients were monitored in a neurosurgical high dependency unit for 24 - 48 hr. Venous blood samples were withdrawn for estimation of serum S100β and plasma NOx (nitrate plus nitrite) concentrations immediately prior to induction of anaesthesia (baseline) (t 0), 10 minutes after aneurysm clipping (t1), 2 (t2), 6 (t3), and 12 hrs postoperatively (t4) and daily thereafter for five days (t5 - 8). Blood samples were centrifuged at 5000 rpm for 5 min and the plasma or serum was separated and stored at -80ºC. Three months after cerebral aneurysm clipping, neurological outcome was assessed using the Glasgow Outcome Scale (GOS)[16] and classified as poor outcome (grade 1 - 3) and good outcome (grade 4 - 5).

**Table 1. Glasgow Outcome Scale for classification of neurological outcome**

| Grade | Description |
|---|---|
| 5 | Good recovery, resumption of life despite minor deficits |
| 4 | Moderate disability (but not dependent) able to travel by public transportation, able to work in public sheltered setting. |
| 3 | Severe disability dependent on others for activities of daily living |
| 2 | Persistent vegetative state |
| 1 | Dead |

***S-100β*** was determined using a commercially available monoclonal two-site sandwich immunoluminometric method LIA-mat® Sangtec® 100 (AB Sangtec Medical, Bromma, Sweden). The sensitivity of this method is < 0.02 μg/L. The sample was incubated with $^{125}$I-labeled monoclonal antibody to S-100β protein. Sample radioactive count rate was measured and compared to that of calibration standards of S-100 β.

***Nitric oxide product*** (NOx) concentrations were measured using a Nitric Oxide Chemiluminescent Analyzer, Sievers 280 NOA™ (Sievers Instruments, Boulder, CO). In solution, NO reacts with molecular oxygen to form nitrite ($NO_2^-$) and with oxyhaemoglobin and superoxide anion ($O_2^-$) to form nitrate ($NO_3^-$). By adding acid and reducing agents, nitrate and nitrite are converted to NO. An inert gas is then used to purge NO from the solution for subsequent detection by chemiluminescence. The detector is based on the reaction of NO with ozone ($O_3$), which produces nitrogen dioxide in an excited state ($NO_2$*) and molecular oxygen. The excited state of $NO_2$* decays to give an infrared chemiluminescence above 600 nm. Light emission is directly related to the NO content of the sample. Sodium nitrate and nitrite were used as standards.

The half-life of no in the body is only a few milliseconds. NO undergoes a series of biological reactions with the final metabolites in vivo being nitrite and nitrate. The relative proportion of nitrite and nitrate is

variable and hence the best reflection of total NO concentration is the sum of both nitrite and nitrate (NOx).

*Statistical Analysis.* Data are expressed as mean ± standard deviation (SD). Differences in concentrations of biomarkers between the defined outcome groups were compared with the Mann -Whitney-U-test. $P < 0.05$ was considered significant.

RESULTS

Fifteen patients (6 Male and 9 Female) mean age 47.4 years (range 32 - 64 years) were studied. Six patients were Grade I, five were Grade II and there were two patients each with Grade III and Grade IV SAH at hospital admission (Table 2).

**Table 2. SAH grade on hospital admission and subsequent GOS at three months after cerebral aneurysm clipping**

| SAH Grade | I | II | III | IV |
|---|---|---|---|---|
| Poor Outcome (GOS) | 1 | 0 | 1 | 2 |
| Good Outcome (GOS) | 5 | 5 | 1 | 0 |

The time from onset of SAH to admission to hospital was 6 - 14 hr. The time from hospital admission to cerebral aneurysm clipping was 24 - 120 hr. At three months postoperatively neurological outcome was good in 11 patients and poor in four patients. Three patients did not have blood samples drawn on the later post-operative days (day 4 - 5) because of transfers to another hospital. Two of these patients were SAH grade II and one was grade I. These patients were included in the GOS scale three-month follow up data. They all were part of the good outcome group.

S100β (μg/L) concentrations were increased at 2 hrs postoperatively (t2) compared to baseline (t0) (0.245 ± 0.04 vs 0.33 ± 0.03)($P < 0.04$). S100β concentrations were also increased compared to base-line at 24 hr postoperatively (t5) (0.245 ± 0.4 vs 0.39 ± 0.3)($P = 0.02$) (Figure 1). There was no difference in S100β concentrations during the study period between the good and poor neurological outcome groups.

NOx (μM) concentrations were less at 12 hr postoperatively (t4) compared to baseline (t0) (9.81 ± 3.25 vs 12.74 ± 2.9)($P < 0.03$) (Figure 2). NOx concentrations were greater on the fourth postoperative day (t7) and fifth postoperative day (t8) compared to baseline (t0) (17.22 ± 7.9, 12.74 ± 2.9 vs 9.81 ± 3.25) ($P < 0.05$). NOx concentrations were greater (fourth post-operative day)(t4) in patients with a poor neurological outcome (n = 4) as compared to the good outcome group (n = 11) (27.3 ± 5.3 vs 11.32 ± 3.3)($P = 0.04$) (Figure 3).

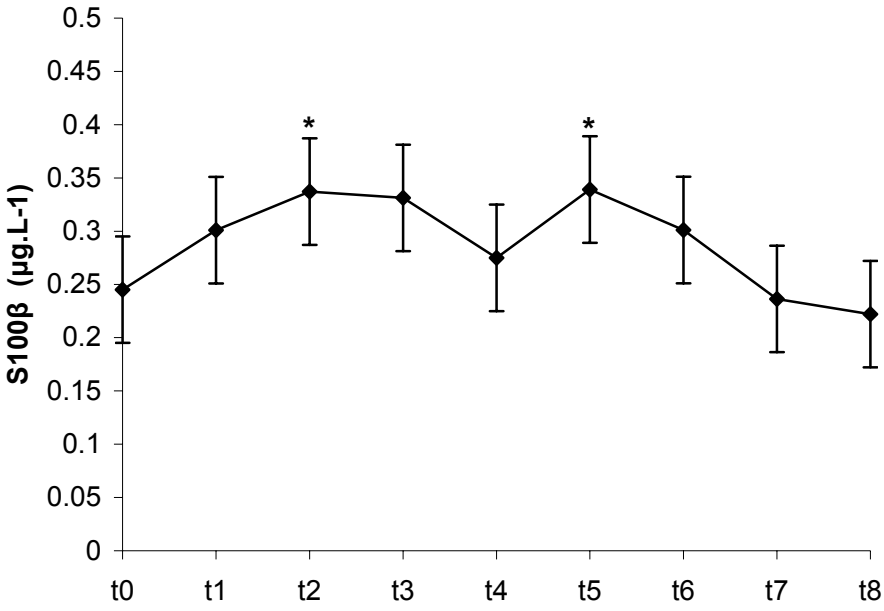


**Figure 1.** S100β (μg/L) concentrations following cerebral aneurysm clipping in patients with subarachnoid haemorrhage. Immediately prior to induction of anaesthesia (baseline) (t 0), 10 minutes after aneurysm clipping (t1), 2 (t2), 6 (t3), and 12 (t4) hrs postoperatively and daily thereafter for five days (t5 – 8).
* $P < 0.05$ compared to baseline. Values are mean ± SD.

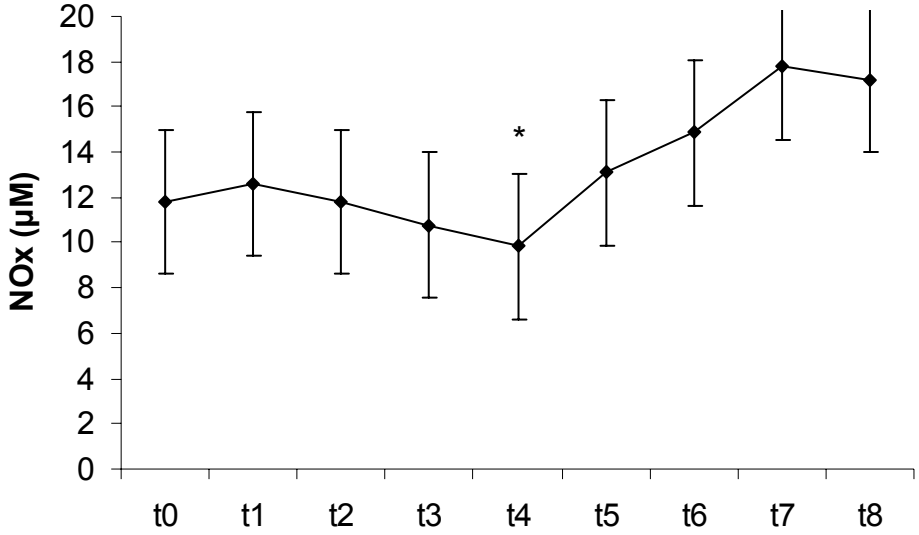


**Figure 2**. NOx (µM) concentrations following cerebral aneurysm clipping in patients with subarachnoid haemorrhage. Immediately prior to induction of anaesthesia (baseline) (t0), 10 minutes after aneurysm clipping (t1), 2 (t2), 6 (t3), and 12 (t4) hr postoperatively and daily thereafter for five days (t5 - 8)
* $P < 0.05$ compared to baseline. Values are mean $\pm$ SD.

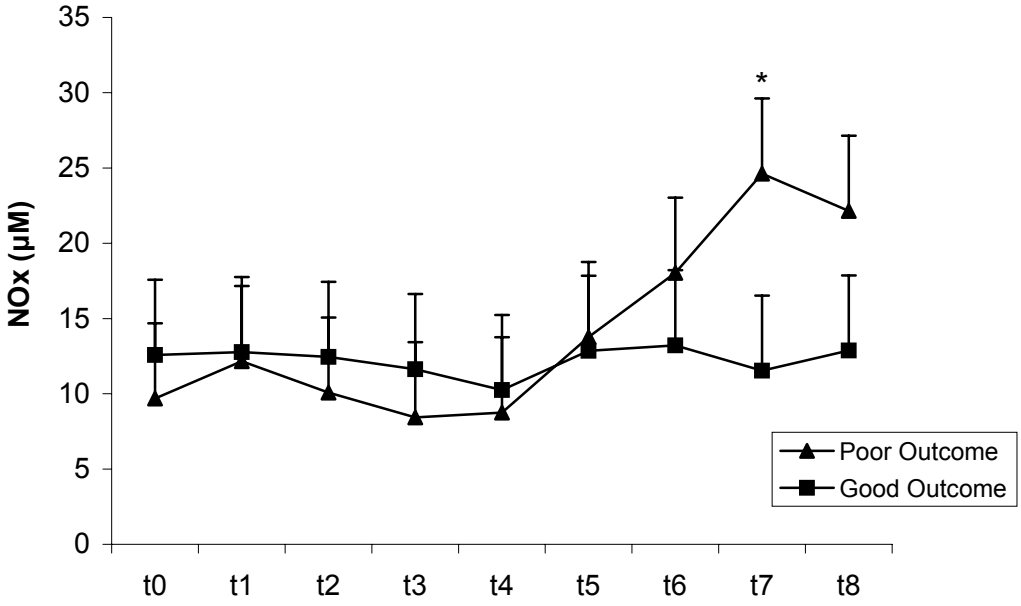


**Figure 3.** NOx (µM) concentrations in patients with Good (n = 11) and Poor (n = 4) neurological outcomes following cerebral aneurysm clipping in patients with subarachnoid haemorrhage. Immediately prior to induction of anaesthesia (baseline) (t0), 10 minutes after aneurysm clipping (t1), 2 (t2), 6 (t3), and 12 (t4) hr postoperatively and daily thereafter for five days (t5 - 8).
* $P < 0.05$. Values are mean $\pm$ SD

## DISCUSSION

This study demonstrates that S100β and NOx concentrations increase following cerebral aneurysm clipping in patients with SAH. The delayed increased NOx concentrations postoperatively were associated with worse neurological outcome.

In this study we found that S100β concentrations increased following cerebral aneurysm clipping in patients with SAH. This has previously not been reported. Persson *et al*,[7] and Wiesmann *et al*,[8] have reported that greater S100β concentrations are associated with increased severity of SAH Grading (defined by GOS) and a poorer neurological outcome following SAH. S100β concentrations are greater in patients with neurological injury after coronary artery bypass surgery.[10,11] Increased S100β concentrations, however, were not associated with the poor neurological outcome group in our study.

In this study we found that NOx concentrations were decreased initially following cerebral aneurysm clipping in patients with SAH. This decrease in NOx concentrations has not been reported previously and may be associated with cerebral vasospasm.[17,18] Intracarotid infusions of NO and NO donors in a primate model of SAH reversed and prevented cerebral vasospasm.[17] After SAH oxyhaemoglobin and deoxyhaemoglobin increase in the peri-vascular space and may act as scavengers for NO.[19] The subsequent increase in NOx concentrations may be due to secondary brain injury caused by vasospasm and/or delayed induction of NOS. However we did not assess the vasopasm during the post-operative period, and are therefore, unable to relate this increase in NOx concentration with the above mechanism(s).

Nitric Oxide plays an important physiological role in various body tissues.[12] The formation of NO is catalysed by the enzyme nitric oxide synthatase (NOS) of which there are three iso-types: type I neuronal NOS (nNOS), type II inducible NOS (iNOS), and type III endothelial NOS (eNOS). The role of NO in cerebral ischaemia is complex. Under the condition of cerebral ischaemia, high concentrations of NO are generated by the calcium-dependent activation of the constitutive neuronal NOS (eNOS and nNOS) and by the activation of the inducible form of NOS (iNOS). Nitric Oxide production is increased at all stages of cerebral ischaemia.[14] The biological characteristics (smooth muscle relaxation) of NO make it a powerful dilator of the cerebral circulation[20] and it has both neuroprotective[21,22] and cytotoxic[14] effects in cerebral ischaemia. The appearance of iNOS after cerebral ischaemia is delayed because, unlike eNOS and nNOS, it is not a constitutive enzyme. Post-ischaemic iNOS induction is likely to be initiated by cytokines that accumulate in the brain and possibly also by local hypoxia.[14] iNOS messenger ribonucleic acid expression begins at 24 to 48 hours after ischaemia and is decreased at 7 days in a rat model of focal cerebral ischaemia.[22] In neuroprotective studies, inhibition of specific isoforms of NOS (for example nNOS) produce more promising results than inhibition of all NOS isoforms.[20]

The primary objective of this study was to characterise the release of markers of neuronal injury (S100β and NOx) after cerebral aneurysm clipping in patients with SAH. Patients were classified as good and poor neurological outcome with 11 and four patients in each group respectively. Thus one of the study limitations was sample size, making definite conclusions difficult regarding associations between concentrations of biomarkers and neurological outcome. In assessing associations between neurological outcome and concentrations of these biomarkers a larger sample size would be required. We did not measure S100β and NOx concentrations in cerbrospinal fluid. Simultaneous estimation of biomarker concentrations in cerebrospinal fluid may increase efficacy.

In conclusion, S100β concentrations increased after cerebral aneurysm clipping in patients with SAH. There was no difference in S100β concentrations during the study period between the good and poor neurological outcome groups. NOx concentrations intially decreased but subsequently increased. Delayed increased NOx concentrations postoperatively were associated with worse neurological outcome.

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