## ORIGINAL ARTICLE

# Effects of sevoflurane and propofol on S100β and neuron-specific enolase protein levels during cardiopulmonary bypass

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## Abstract

**Aim:** Cardiopulmonary bypass (CPB) is associated with the release of S100β and neuron-specific enolase (NSE) indicating cerebral cell injury. The purpose of the present study was to evaluate the effect of propofol and sevoflurane on S100β and NSE levels in patients undergoing coronary artery bypass grafting (CABG).

**Materials and Methods:** Twenty male patients undergoing CABG were randomly allocated into two groups. One group received sevoflurane (GS) and the other received propofol (GP). Arterial blood samples for analysis of S100β and NSE levels were taken preoperatively (T1), 30 min after initiation of CPB (T2), at the end of CPB (T3), 1 (T4), 6 (T5) and 24 h (T6) postoperatively.

**Results:** S100β level was significantly higher compared to all analyzed times at T3 in both groups ($P < 0.001$). S100β level was significantly higher in GP than GS only at T2 ($P = 0.002$). NSE level was significantly higher at T3, T4 and T5 than T1 in the GP ($P = 0.001$, 0.002 and 0.023, respectively), while a significant increase was seen at T3 and T4 in GS group ($P = 0.001$ and 0.047, respectively).

**Conclusion:** Our findings showed that both S100β and NSE levels similarly increased during CPB and immediately after CPB during sevoflurane and propofol based anesthesia.

**Key words:** Cardiopulmonary bypass, neuron-specific enolase, propofol, S100β, sevoflurane

## Introduction

Despite technological developments in cardiopulmonary bypass (CPB), improvements in surgical techniques and better anesthesia management, hypoxic brain damage remains a common and serious complication of cardiac surgery. The pathogenesis of cerebral injury during cardiac operations is unclear and multifactorial. Hypoxia, patient age, procedure type, CPB duration, and aortic cross-clamp have been implicated in cerebral injury.[1-3] Pre- and post-operative clinical and neuropsychological examinations, computed tomography or magnetic resonance imaging can be used to diagnose cerebral dysfunction postoperatively. However, these procedures may not be convenient for patients immediately after a cardiac surgery. Therefore, the use of biochemical markers to detect and quantify cerebral injury may be of relevant practical value. S100β protein and neuron-specific enolase (NSE) have been evaluated as valid serological markers for cerebral injury.[1,4-8]

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S100β protein is an acidic, calcium-binding protein with a molecular weight of approximately 21 kDa. S100β subunits are present in high concentrations in the extracellular space and cytoplasm of glial and Schwann cells.[9] S100β is eliminated by the kidney with a biological half-life of about 2 h.[10] Elevated concentration of S100β in serum indicates both neuronal damage and increased permeability of the blood-brain barrier.[11]

Neuron-specific enolase is an intracytoplasmic glycolytic enolase enzyme that is localized in neurons. When neuronal damage has occurred, NSE is released into the cerebrospinal fluid and cerebral and systemic circulation.[7] NSE has a biological half-life of about 24 h. Ramlawi *et al.* reported that NSE may be a better serum marker of brain injury following CPB than S100β.[4]

Neuroprotective properties of anesthetic agents have been previously reported.[12] Sevoflurane improved neurological outcomes after incomplete cerebral ischemia in rats, and it was neuroprotective during focal or global cerebral ischemia.[13,14] Established *in vitro* and *in vivo* experimental models of mild/moderate acute cerebral ischemia have shown that propofol (2,6-diisopropylphenol) has a neuroprotective effect related to the decrease in cerebral metabolic rate for oxygen.[15]

Two studies by Kanbak *et al.* examined the effects of anesthetic agents on neurocognitive outcomes and S100β protein levels in patients undergoing coronary artery bypass grafting (CABG).[16,17] In the first study, they concluded that propofol appeared to offer no advantage over isoflurane for cerebral protection.[16] In the second study, they reported that higher S100β levels were seen with sevoflurane compared to isoflurane and desflurane.[17]

Until, there has not been a study comparing the effects of propofol and sevoflurane on S100β and NSE levels during and after CPB surgery. The purpose of the present study was to evaluate the effect of propofol and sevoflurane anesthesia on S100β and NSE levels in patients undergoing CABG.

## Materials and Methods

After obtaining informed consent and approval from the Ethics Committee of the Medical Faculty of Ataturk University. 20 male patients below 70 years of age undergoing elective CABG were selected for this study. Patients with heart failure or poor ventricular function (defined by an ejection fraction <50%), neurological disease, diabetes mellitus, renal failure (serum creatinine >2.0 mg/dL), atheromatosis or calcification in the ascending aorta evaluated by Doppler ultrasonography, additional valve disease or reoperation were excluded from the study. All patients had normal sinus rhythms.

All patients were premedicated with oral diazepam (10 mg) the night before the surgery. Patients were randomly assigned to two groups with MedCalc statistical software; one receiving propofol ($n = 10$) and the other receiving sevoflurane ($n = 10$). All patients received midazolam (0.1 mg/kg) and fentanyl (5 µg/kg) for anesthesia induction and were paralyzed with vecuronium bromide (0.12 mg/kg). Sevoflurane or propofol was used for maintenance of anesthesia depending on the patient group. Sevoflurane was administered by inhalation at 1–1.5% before and after CPB and 0.25–0.5% during CPB. Propofol was infused at 6–10 mg/kg/h before and after CPB and 3 mg/kg/h during CPB. Fentanyl infusion (10 µg/kg/h) was used in all patients during surgery and 40–70% $O_2$ in air was used during the maintenance of anesthesia. Bispectral index (BIS) was used to monitor the depth of the anesthesia and the anesthetics were titrated in order to keep patient BIS between 40 and 60.

In all cases, electrocardiogram, radial or brachial arterial pressure, central venous pressure, and nasopharyngeal temperature were continuously monitored. All surgical procedures were performed by the same surgical team. Surgery was performed through a median sternotomy. Unfractionated heparin (3 mg/kg) was administered before initiating CPB to maintain an activated clotting time of more than 450 s. CPB was initiated after cannulation of the right atrium and ascending aorta. Perfusion was performed with a roller pump (Cobe Industries, Denver, CO, USA), a membrane oxygenator (Cobe Industries, Denver, CO, USA), and arterial line filter. Perfusion flow was nonpulsatile with a flow rate of 2.4 L/min/m² at approximately 30°C. Cardiac arrest was achieved by infusion of cold (4°C) cardioplegia solution (Plegisol) anterogradely. Blood cardiology was repeated every 30 min. The alpha-stat strategy was used for $PaCO_2$ and pH management during CPB. The mean arterial pressure was maintained at a target range of 40–60 mmHg with epinephrine or nitroglycerine if needed. Hematocrit during CPB was maintained between 22% and 26%. The heparin effect was reversed with protamine sulfate (1.2 mg protamine sulfate per 100 IU heparin) at decannulation. The left internal thoracic artery was the chosen graft for left anterior descending coronary artery and radial artery or saphenous vein graft for the other anastomosis. After having completed distal anastomosis with aortic cross-clamping, the proximal conduits were finalized behind a side aortic clamp. Following surgery, patients were transferred to an intensive care unit, and they were weaned from mechanical ventilation and extubated. Arterial blood samples for analysis of S100β and NSE proteins were taken at the following six time points: Preoperatively after induction of anesthesia to establish baseline levels (T1), 30 min after initiation of CPB (T2), at the end of CPB (T3), and 1 (T4), 6 (T5) and 24 h (T6) postoperatively. S100β was assayed by a sandwich enzyme-linked immunoabsorbant assay (S100β ELISA, BioVendor, Modrice, Czech Republic). This assay is

as sensitive as the Sangtec assay but has greater specificity and does not cross-react with the S100β monomer found in the heart, aorta, and mediastinal fat.[18,19] An ELISA assay was also used for NSE (NSE ELISA, DRG International Inc., USA). Samples were transferred to lithium heparin tubes and immediately centrifuged at room temperature (4000 rpm for 5 min) to separate the plasma. The plasma was then frozen at $-20°C$ and stored for later analysis. The limits of detection were 5 pg/L for S100β and 0.03 µg/L for NSE.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 15.0 software for Windows (SPSS Inc., Chicago, IL, USA). The Student's *t*-test was used to compare parametric data, including demographic characteristics, ejection fraction, duration of anesthesia, duration of surgery, CPB time, and aortic cross-clamp time. A two-way ANOVA test was used to test repetitive measurements of S100β and NSE levels. $P < 0.05$ was considered to be statistically significant. S100β and NSE levels are presented as mean ± standard deviation. This study was designed with the same patient numbers as Kanbak's study.[16]

## Results

Ten patients for each group were enrolled in the study. Myocardial infarction, perioperative stroke or death did not occur in any patients during the operation. The patients were transferred to intensive care unit and after remaining under close monitoring for 48 h in the care unit they were transferred to the department of cardiovascular surgery. The patients' demographic properties, ejection fraction, duration of anesthesia and surgery, and CPB and aortic cross-clamp time were similar in both groups [Table 1].

### S100β

The measured levels of S100β are shown in Figure 1a-c. In both groups, S100β levels increased in all the blood samples taken throughout the surgical procedure and postoperative phase. S100β reached a maximum at the end of CPB (T3) in both groups. When the levels of S100β were compared between groups at the same sampling times, the only significant difference was at 30 min of CPB (T2), higher in propofol group than sevoflurane group ($P = 0.002$). The level of S100β for both groups was significantly higher at T3 compared to all other times ($P < 0.05$).

In the sevoflurane group, the S100β levels at 30 min of CPB (T2) were not significantly higher than baseline (T1), 6 (T5) or 24 h (T6) postoperative values ($P < 0.05$). In the propofol group, S100β levels at 30 min of CPB (T2) were significantly higher than baseline (T1), 6 (T5) or 24 h (T6) postoperative values ($P < 0.001$ for all). S100β levels were significantly higher 1 h postoperatively (T4) than baseline in the sevoflurane group ($P = 0.038$) and in the propofol group ($P = 0.001$). S100β levels 1 h postoperatively were significantly higher than 6 or 24 h postoperatively ($P = 0.014$ and 0.004, respectively) in the propofol group, while there was no significant difference in the sevoflurane group ($P = 0.295$ and 0.090, respectively).
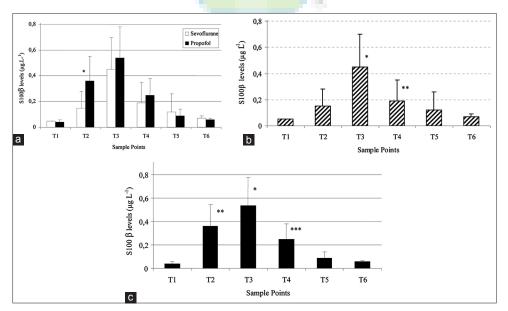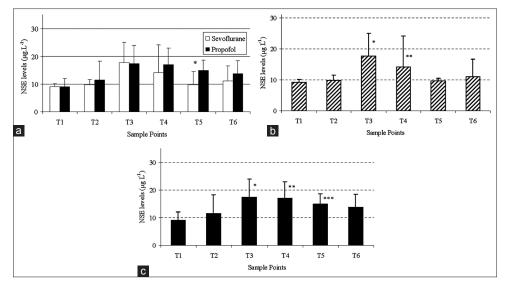


**Figure 1**: (a) S100β levels of two groups at analyzed time points. S100β levels were significantly higher in group propofol than group sevoflurane only at T2 (*$P = 0.002$). (b) S100β levels of group sevoflurane at analyzed time points. S100β levels at T3 were significantly higher compared to all analyzed times (*$P < 0.05$ for all compares) and S100β levels at T4 were significantly higher than T1 (**$P = 0.038$). (c) S100β levels of group propofol at analyzed time points. S100β levels at T3 were significantly higher compared to all analyzed times (*$P < 0.001$ for all compares), S100β levels at T2 were significantly higher than T1, T5 and T6 (**$P < 0.001$) and S100β levels at T4 were significantly higher than T1, T5 and T6 (***$P < 0.05$). Values are expressed as mean ± SD. T1 = After induction of anesthesia (baseline), T2 = 30 min of CPB, T3 = At the end of CPB, T4 = 1 h postoperatively, T5 = 6 h postoperatively and T6 = 24 h postoperatively

**Figure 2:** (a) Neuron-specific enolase (NSE) levels of two groups at analyzed time points. NSE levels were significantly higher in group propofol than group sevoflurane only at T5, (*$P$ = 0.046). (b) NSE levels of group sevoflurane at analyzed time points. NSE levels at T3 were significantly higher compared to T1, T2, T5 and T6 (*$P$ < 0.05 for all compares) and NSE levels at T4 were significantly higher than T1 (**$P$ = 0.047). (c) NSE levels of group propofol at analyzed time points. NSE levels at T3 were significantly higher compared to T1 and T2, (*$P$ < 0.05 for all compares), NSE levels at T4 were significantly higher than T1 and T2 (**$P$ < 0.05) and NSE levels at T5 were significantly higher than T1 (***$P$ < 0.05). Values are expressed as mean ± SD. T1 = After induction of anesthesia (baseline), T2 = 30 min of CPB, T3 = At the end of CPB, T4 = 1 h postoperatively, T5 = 6 hpostoperatively and T6 = 24 h postoperatively

### Table 1: Patient demographic and clinical characteristics

|  | Group S $n=10$ | Group P $n=10$ | $P$ |
|---|---|---|---|
| Age (years) | 60.0±7.4 | 57.8±2.3 | 0.512 |
| Weight (kg) | 72.9±15.5 | 69.9±10.5 | 0.619 |
| Height (m) | 1.66±0.05 | 1.64±0.07 | 0.503 |
| Ejection fraction (%) | 59.0±6.9 | 56.2±5.1 | 0.322 |
| Duration of anesthesia (min) | 275.0±36.6 | 285.0±76.8 | 0.618 |
| Duration of surgery (min) | 247.5±40.3 | 258.5±76.5 | 0.519 |
| CPB time (min) | 116.1±15.4 | 100.5±22.5 | 0.087 |
| Aortic cross-clamp time (min) | 68.7±11.4 | 58.9±17.1 | 0.150 |

Values are expressed as mean±SD. SD=Standard deviation; CPB=Cardiopulmonary bypass

In both groups, increased S100β levels were observed at 6 h postoperatively compared to after induction of anesthesia but the difference was not significant. S100β levels decreased nearly to baseline levels at 24 h postoperatively in both groups.

### Neuron-specific enolase
The measured levels of NSE are shown Figure 2a-c. NSE levels were higher postoperatively in both groups. When NSE levels were compared between the two treatment groups, the NSE levels in the propofol group were higher than the sevoflurane group except for the levels at the end of CPB (T3). However, this difference was significant only at 6 h postoperatively (T5) ($P$ = 0.046).

Neuron-specific enolase increased to maximum levels at the end of CPB (T3) in both groups. These levels were

significantly higher than baseline (T1), at 30 min of CPB (T2), 6 (T5) or 24 h (T6) postoperative levels in the sevoflurane group ($P$ < 0.05). However, it is significantly higher than baseline (T1) and at 30 min of CPB (T2) in the propofol groups ($P$ < 0.05). The levels of NSE were also significantly higher compared to baseline levels (T1) at 1 (T4) and 6 h (T5) postoperatively in the propofol group ($P$ = 0.002 and 0.023, respectively). In the sevoflurane group, significantly higher levels were seen 1 h postoperatively compared to baseline levels ($P$ = 0.047).

## Discussion

In this study, we determined the effects of sevoflurane and propofol based anesthesia on serum S100β and NSE levels during and after CPB. It was shown that both S100β and NSE levels increased during CPB and immediately after CPB for both sevoflurane and propofol anesthesia.

It is well-known that CPB is associated with the release of brain-specific S100β and NSE proteins indicating cerebral cell injury. Basile *et al.* reported that perioperative release patterns of both S100β and NSE predict persistent cerebral damage after cardiac surgery.[6] There are similarities and differences in the S100β and NSE levels reported in Basile's study and the work presented here. Basile *et al.* reported that both proteins were increased in all the blood samples taken throughout the surgical procedures and the postoperative phases However, S100β levels remained significantly higher 48 h after surgery compared to baseline in Basile's study, while in our study S100β levels were not

significantly different from baseline 24 h after surgery in both groups. NSE levels also remained significantly elevated 24 h after surgery in Basile's study, while in our study levels were not significantly different from baseline in both groups. Basile's study indicated that both S100β and NSE levels increased during cardiac surgery and showed the level of variation was related to the impairment in selective neuropsychological performances assessed 6 months after surgery, which possibly expressed persistent cerebral injury. Unfortunately, no neuropsychological testing was performed in our study.

In the literature, there are only two reports that examine the effects of different anesthetic techniques on the release of S100β and cognitive outcomes associated with CPB.[16,17] Both studies were reported by Kanbak *et al*. In the first study, they evaluated the effect of propofol and isoflurane on neuropsychological test performance and S100β protein levels in patients undergoing CABG.[16] They expected to have lower S100β protein levels in the propofol group than the isoflurane group because of the neuroprotective action of propofol. However, S100β protein levels were higher in the propofol group. S100β protein levels peaked at the end of CPB and returned to baseline levels at 24 h postoperatively in both groups. However, the increase was not significant in the isoflurane group. In our study, S100β and NSE protein levels peaked at the end of CPB in both groups, and the levels of both proteins were not significantly higher from baseline levels in both groups 24 h postoperatively.

In the second study reported by Kanbak *et al*., the effect of isoflurane, sevoflurane and desflurane on postoperative cognitive outcomes, and S100β protein levels were compared.[17] They concluded that isoflurane was associated with better neurocognitive functions than desflurane and sevoflurane, and prolonged brain injury indicated by high S100β levels seen with desflurane. Also, they found that levels of S100β increased during CPB and then decreased to baseline levels on postoperative 1st day in the sevoflurane group, but remained higher on the 3rd and 6 days postoperatively in the desflurane group. In our study, the levels of both proteins were not significantly from baseline levels in both groups 24 h postoperatively.

Most anesthetic agents have neuroprotective effects due to their ability to reduce the cerebral demand for oxygen. This has a beneficial impact on the balance between brain energy supply and demand, resulting in increased neuronal tolerance to hypoxic/ischemic injury.[15] Propofol has a number of different neuroprotective effects. Propofol reduces cerebral metabolic rate and cerebral blood flow and results in suppression of brain electrical activity.[20,21] Propofol also attenuates glutamate-mediated excitotoxicity by decreasing NMDA receptor activation, reducing glutamate release, or recovering the function of transporters responsible for glutamate uptake into neuronal and glial cells.[15] Sevoflurane also has neuroprotective effects. Activation of mitochondrial $K_{ATP}$ channels during the trigger and effector phase is a key mechanism of sevoflurane induced preconditioning.[22] Preconditioning of the brain against ischemic injury might be a strategy to reduce morbidity and mortality in patients when ischemic episodes are anticipated during cardiac surgery. The propofol group was expected to have less S100β and NSE levels than the sevoflurane group because of the brain protection of propofol. However, unexpectedly, it was found that S100β and NSE levels were similar in two groups. Based on the changes in S100β and NSE levels measured here, we think propofol protects the brain from neuronal damage induced by brain ischemia, and sevoflurane appears to precondition the brain against anticipated ischemic injury resulting from hypoperfusion during CPB.

Our study included a small number of patients. In future studies, greater patient numbers should be used and neuropsychological tests should be used in addition to measuring S100β and NSE levels, and also it will be better to investigate the correlation between clinical outcome and S100β and NSE levels.

## Conclusion

Our findings showed that both S100β and NSE levels increased during CPB and immediately after CPB during sevoflurane and propofol based anesthesia. The elevation observed in both groups was similar at most time periods. Both S100β and NSE were higher in the propofol group than the sevoflurane group at one of the six measurement times; however, the peak levels of both markers were not significantly different between the groups.

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