Neuronal and Astroglial Injuries in Patients Undergoing Coronary Artery Bypass Grafting and Aortic Arch Replacement During Hypothermic Cardiopulmonary Bypass

Dmitry N. Derkach, MD, Hirotugu Okamoto, MD, PhD, and Shosuke Takahashi, MD, PhD
Department of Anesthesiology and Critical Care Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan

More than 50% of patients suffer neuropsychologic impairment after cardiac surgery. We measured neuron-specific enolase (NSE) and S-100 protein (S-100) in patients’ serum as putative markers of neuronal and astroglial cell injury, respectively. Group I (n = 13) underwent coronary artery bypass grafting (CABG) with mild hypothermic cardiopulmonary bypass (CPB); Group II (n = 6) underwent aortic arch replacement with deep hypothermic CPB; Group III (n = 8) underwent CABG under normothermia without CPB. During and after the operation, serum levels of NSE and S-100 were significantly increased only in Groups I and II (during CPB), NSE still being increased 12 h after surgery in Group II. This suggests that neuronal and astroglial cell injuries are more likely in patients undergoing CABG with mild hypothermic CPB or aortic arch replacement with deep hypothermic CPB than in those undergoing CABG under normothermia without CPB. However, these increases of NSE and S-100 failed to reflect clinical brain damage. Rather, an electroencephalogram, was only capable of detecting neurologic complications after surgery.

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Address correspondence to Hirotsugu Okamoto, MD, PhD, Department of Anesthesiology, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara, Kanagawa, 224-8555, Japan. Address e-mail to okasuke@med.kitasato-u.ac.jp.

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in Kyushu University Hospital. Informed consent was obtained from each patient before the operation. Patients with a history of head trauma, seizures, or brain tumors were excluded. Twenty-seven patients were divided into three groups according to the type of surgery: Group I had 13 patients undergoing CABG with mild hypothermic CPB, Group II had 6 patients undergoing AAR with deep hypothermic CPB and retrograde cerebral perfusion, and Group III had 8 patients undergoing CABG without CPB under normothermic conditions (beating heart surgery). Four other patients were excluded from the research protocol because we failed to localize the catheter tip in the bulb of the internal jugular vein (2 patients) or because severe hemolysis occurred (2 patients) during CPB.

All patients were premedicated with nitrazepam 5–7 mg 2 h before surgery. Anesthesia was induced with fentanyl (0.01–0.02 mg/kg) and diazepam (0.2 mg/kg). After endotracheal intubation aided by pancuronium (0.1 mg/kg), fentanyl (0.25–0.5 mg/h) and diazepam (1.0 mg/h) were used for the maintenance of anesthesia. The extracorporeal circuit consisted of a membrane oxygenator (D 903 Avant™, Dieco, Mirandola, Italy), silicon tubes, a reservoir, and an arterial filter. Acetated Ringer’s solution supplemented with 60 mEq of bicarbonate, 40 g of mannitol, and 200 mL of 25% albumin per liter was used to prime the circuit. Hydrocortisone 50 mg/kg, heparin 3 mg/kg, and tranexamic acid 50 mg/kg were given before aortic cannulation. CPB was performed by using nonpulsatile perfusion during mild hypothermia in Group I (arterial blood temperature from oxygenator 28°C, nadir PA blood temperatures (29°C–32°C) and during deep hypothermia in Group II (arterial blood from oxygenator 16°C, nadir PA blood temperature 16°C–20°C). The pumpflow rate and the retrograde cerebral perfusion flow rate were maintained at 2.2–2.5 L·min⁻¹·m⁻² and 0.3–0.5 L·min⁻¹·m⁻² respectively. Acid-base management during CPB was achieved by using an alpha-stat strategy, and mean arterial blood pressure was maintained at 50–80 mm Hg. In Group III, aortic and venous cannulations for CPB were performed as in Groups I and II, but the CABG was performed without CPB under normothermic conditions.

Monitors included TCD (Mediasonics, Fremont, CA), NIRS (INVOS 3100A Cerebral Oximeter™; Somanetics, Troy, MI) and EEG (Nihonkohden, Tokyo, Japan). $S_{O_2}$ (Oxymetrix-3 SO₂/CO Computer™; Abbott Critical Care Systems, Mountain View, CA) was measured by way of a catheter (5.5F) positioned with the aid of radiographic examination in the bulb of the internal jugular vein. $S_{O_2}$ measurements were calibrated by gas analysis of samples taken from the distal port. Monitoring of TCD was unilateral (right side). After intubation, TCD detector was adjusted in the projection of the middle cerebral artery and fixed in the position with highest signal amplitude. NIRS monitoring was bilateral with sensors fixed on both right and left sides of forehead.

Standard preoperative and postoperative neuro- assessments were performed by neurologists for all patients. The standard neuro-assessment consists of testing sensory and motor nerve function, cranial nerve function, deep tendon reflexes of extremities, pathetic reflexes, and mental (neuropsychological) status by conducting brief calculations and conversation. Computed tomography examination (CT) was available for patients with perioperative clinical evidence of neurological deficit or seizures.

Blood samples for NSE, S-100, and lactate analysis were drawn from the distal port of the $S_{O_2}$ catheter. Jugular venous and arterial lactate concentrations were determined by using a lactate and glucose analyzer (2300™; YSI, Yellow Springs, OH). Blood 5 mL for NSE and S-100 analysis was centrifuged for 10 min, and serum was frozen until assayed. For NSE content determination, the sandwiched immunoradiometric assay system described by Johnsson et al. (7) was used (NSE RIA kit™; Eiken, Tokyo, Japan). Standard NSE and samples were incubated for 90 min with anti-NSE antibody beads and $^{125}$I-labeled anti-NSE antibody. After washing out excess $^{125}$I-labeled materials, the amount of radioactive antibody was measured by using a gamma counter. S-100 was analyzed by using the enzyme immunoassay originally described by Usui et al. (9). After immobilizing anti-S-100 antibody using a microplate, standard S-100 and samples were incubated overnight with peroxidase-labeled anti-S-100 antibody, and the chemiluminescence was then measured.

All results were expressed as mean ± SEM. For all the measured variables, an analysis of variance was used to compare the changes among groups, and when significance was detected, a Student’s $t$-test with Bonferroni’s corrections was used to compare pairs of measured points. The patients’ demographic data and clinical outcome were analyzed among the groups by using Friedman’s significance test. Statistical significance was set at $P < 0.05$.

**Results**

There were no significant differences in demographic and operative characteristics among the groups (Table 1). As indicated in Table 2, eight patients had carotid stenosis (left, right or both carotid arteries impaired), and three patients had a history of stroke. As shown in Figure 1A, the patients in Group I (CABG with mild hypothermic CPB) or Group II (AAR with deep hypothermic CPB) exhibited a significant increase in serum NSE compared with the patients in Group III (CABG without CPB from 90 min after the
The NSE level was still increased in Group II 12 h after the end of surgery, but it had returned to normal in the patients in Group I. The S-100 concentration (Fig. 1B) was significantly larger in Groups I and II than in Group III from 90 min after the beginning of CPB until the end of CPB, and it had decreased to within the normal range (less than 0.5 mg/L) within 12 h of the end of the operation. In Group III, NSE and S-100 both stayed within the normal range throughout the study (Fig. 1, A and B). The jugular venous-arterial difference in lactate concentration is shown for each group in Figure 2. There were no differences among the groups throughout the operative period. At 90 min after starting CPB, SjO2 in Group II was higher (99.6% ± 0.2%) than in Group I (74.4% ± 3.6%) or Group III (66% ± 1.7%) (Fig. 3) because of the use of retrograde cerebral perfusion. At some measurement points, NIRS gave higher values in Group I and Group II than in Group III; however, there was no consistent pattern.

### Table 1. Demographic and Operative Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>CPB (min)</th>
<th>Aortic clamping time (min)</th>
<th>CI (L·min⁻¹·m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: CABG</td>
<td>12M, 1F</td>
<td>66 ± 3</td>
<td>62 ± 2</td>
<td>161 ± 2</td>
<td>161 ± 12</td>
<td>92 ± 12</td>
<td>3.4 ± 0.4</td>
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<tr>
<td>II: AAR</td>
<td>4M, 2F</td>
<td>63 ± 4</td>
<td>62 ± 5</td>
<td>159 ± 5</td>
<td>265 ± 26</td>
<td>100 ± 41</td>
<td>3.7 ± 0.1</td>
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<tr>
<td>III: beating heart surgery</td>
<td>7M, 1F</td>
<td>63 ± 3</td>
<td>65 ± 3</td>
<td>164 ± 3</td>
<td>—</td>
<td>—</td>
<td>3.2 ± 0.1</td>
</tr>
</tbody>
</table>

Data are number of patients or mean ± se. AAR = aortic arch replacement, CI = cardiac index before operation, CPB = cardiopulmonary bypass, CABG = coronary artery bypass grafting.

### Table 2. Perioperative Data and Clinical Outcome

<table>
<thead>
<tr>
<th>History of stroke</th>
<th>Carotid stenosis</th>
<th>Seizures during oper.</th>
<th>1st day in ICU</th>
<th>Recovery of consciousness (day in ICU)</th>
<th>Days in ICU (total)</th>
<th>Neurological status on the 3rd p/o day</th>
</tr>
</thead>
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<tr>
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</table>

AAR = aortic arch replacement, CABG = coronary artery bypass grafting, R = right, L = left, CI = computed tomography.

*No clinical evidence of stroke, but CT abnormality was detected.*

*Patient with confusion mental state.*

*N. abducens sinistrae insufficiency, suspected brain stem stroke; no abnormality in CT.*

*Transient right side paralysis, no abnormality found in 6th day CT.*
Cerebral blood flow velocity measured by TCD was found to be variable (Fig. 4). In all three groups. In the EEG, two patients in Group I and one patient in Group II developed a seizure pattern at the end of the operation. All the other patients in the three groups showed a pattern (low amplitude and frequency) throughout the operation that was typical of patients under anesthesia.

All the patients recovered after their operation. As shown in Table 2, four patients two from Group I [15.4%] and two from Group II [33.3%] sustained seizures either at the end of the operation or the day after the operation. Concomitant decreases in the values of NIRS and \( S_j \) were observed, but not in TCD. Bolus IV injections of thiamylal 100 mg and midazolam 5 mg were given, and continuous infusions of the same dosages of the drugs per hour were started. These patients required a more prolonged period of mechanical ventilation and hence regained consciousness later than the other patients (1–4 days) after the operation. For several days, they were confused, restless, or agitated and therefore spent more days in the intensive care unit. One had respiratory problems in the postoperative period, and a tracheostomy was performed on the 15th postoperative day. On the 3rd postoperative day, one patient in Group I (7.7%) and one patient in Group II (16.7%) showed evidence of a neurological deficit (Table 2). In contrast, no patients in Group III had postoperative neurological complications. However, statistically, there were no significant differences among the three groups in terms of postoperative neurological outcome.
mean NSE level was larger than 30 μg/L in Group II patients (n = 6, with deep hypothermic CPB), and it remained more than 30 μg/L even 12 hours later. In Group I, the NSE was increased to 20–30 μg/L (which could be considered borderline levels). In contrast, the control group (Group III) showed no increases. Our data suggest that patients in Groups I and II may have had neuronal cellular injury, even though the injury may have been subclinical in some cases.

S-100 protein is an acidic calcium-binding protein found in the brain as homodimers of two isomeric subunits, α and β. S-100β (ββ-S-100) is present in large concentrations in glial cells and Schwann cells, whereas S-100α (αβ-S-100) is present in glial cells, but not in Schwann cells (14). S-100 protein is considered a marker of glial cell damage. Clinical data suggest that the normal level of serum S-100 is less than 0.2 μg/L (15). We set our normal range at less than 0.5 μg/L, the difference in normal values being explained by differences in the assay protocols. In our study, the greatest increase in serum S-100 protein was seen in patients undergoing AAR on weaning from deep hyperthermic CPB, and the level had returned to normal within 12 hours of the end of the operation (Fig. 1B). In patients of Group I (CABG with mild hypothermic CPB), the increase in S-100 was moderate. The time course of the present changes in S-100 is consistent with that in a previous report (15), and we confirmed that S-100 protein is a short-term biochemical marker by comparison with NSE (Fig. 1A). Indeed, the optimal sampling points are not later than 2–3 hours after potential cerebral damage. These NSE increases during cardiac surgery are consistent with the observations of Johnson et al. (7). Our data suggest that astroglial injuries are more likely in patients under mild or deep hypothermic CPB than in patients without CPB under normothermic conditions.

However, several factors should be considered when interpreting data such as ours. First, it is well known that NSE is affected by hemolysis because it is contained within red cells and platelets. To avoid this hemolytic effect, we excluded patients with severe hemolysis. However, we cannot eliminate the possibility that subclinical hemolysis explains the differences. Second, hypothermia itself enhances the washout of these enzymes from the brain (16). We consider that the levels of NSE and S-100 seen in Group II are too high to be leakage from the brain at normal turnover rates. However, it is equally likely that these results may reflect changes in the kinetics of these enzymes as a result of changes in blood-brain barrier permeability during CPB. Third, two patients in Groups I and II suffered neurological complications. These patients had NSE and S-100 levels such as 25 and 23 μg/L, 5.09 and 1.7 μg/L, respectively. Only one of these results (5.09 μg/L) is outside of the two
standard deviations of the entire values of Group I. Another two patients in Group II who developed sei-
zures had levels of NSE and S-100 of 58 and 45 μg/L as well as 5.5 and 2.0 μg/L, respectively. These values are within the range of a standard deviation for the group values. Therefore, we failed to find any corre-
lation between seizure episodes or neurological comp-
lications and the increases in NSE or S-100. It is possible that the small numbers of patients in our study precluded correlations. There may also be other explanations for this. In the central nervous system, different anatomical areas are responsible for different functions, and these differ widely in their structural characteristics. Hence, damage to the same number of neurons in the frontal lobe or temporal lobe (for example) will lead to different levels of neurological impairment. Nonetheless, our data suggest that the number of damaged brain cells was neglectible in the patients not exposed to CPB because there were no increases in either NSE or S-100. This seems to be supported by the neurological outcome: no patient in this group sustained seizures or had neurologic comp-
lications in the perioperative period. In this sense, CABG without CPB under normothermia might be considered in patients with preexisting severe cerebro-
vascular disease. We performed brief neuropsycholo-
getic test that consisted of conversation and calcula-
tion and found no impairments in any patients. However, this test is not sufficient to detect subtle changes in neuropsychologic status, and therefore, other testing based on the more detailed interview (high cognitive function testing) should be performed in future studies.

We also simultaneously used the available intra-
operative monitors/markers (NIRS, TCD, EEG, Sjo2, 
Sj lactate) which are considered real-time instru-
ments for detecting brain damage. Among these (Table 2), all patients with abnormal neurological examination on the third day and seizures in the intensive care unit were predicted by intraoperative EEG monitoring. It is therefore suggested that the EEG is a far more sensitive and specific measure for clinical and neurological dysfunction than NSE and S-100. NIRS and Sjo2 are monitors used for evaluating the balance between cerebral blood flow and cerebral oxygen consumption, and both are useful during cardiac operations (17). Our NIRS values could have been affected by extracranial factors such as skin blood flow or hemoglobin levels, which vary with temperature and CPB, and the Sjo2 is affected by the retrograde cerebral perfusion. In addition, although both NIRS and Sjo2 can detect a global oxygen imbalance sufficient to lead to a se-
vere neurological deficit, they cannot detect local-
ized or mild ischemia such as might occur during CPB. Arterial-jugular vein lactate difference is an-
other biochemical marker of cerebral ischemia that is used in cardiac operations (6). However, this marker is not sufficiently sensitive to detect a tran-
sient anaerobic state in brain cells (18) such as might have occurred in our study. TCD is a useful monitor of cerebral hypoperfusion in patients undergoing cardiac surgery (19). In our study, there were no significant differences in TCD data among the groups, and therefore, the presumed brain cell dam-
age in our patients was probably not a result of changes in the velocity of blood flow in the middle 
cerebral artery.

In summary, during mild or deep hypothermic 
CPB, damage to both neuronal and glial cells likely 
occur. Further, our results suggest that patients under-
going CABG without CPB under normothermic condi-
tions are less likely to suffer brain cell injury.

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