SURGERY FOR CONGENITAL HEART DISEASE

SERUM S-100 PROTEIN LEVELS AFTER PEDIATRIC CARDIAC OPERATIONS: A POSSIBLE NEW MARKER FOR POSTPERFUSION CEREBRAL INJURY

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Background: The release of neuron-specific astroglial S-100 protein to the cerebrospinal fluid is a marker of cerebral damage. The aim of this study was to determine the pattern of release of S-100 protein to serum after pediatric cardiac operations and extracorporeal circulation. **Methods:** Sequential blood samples from 97 children (up to 16 years) were taken after induction of anesthesia, immediately after the discontinuation of extracorporeal circulation, and 5 and 15 hours after extracorporeal circulation. The children were divided into five groups including three age groups, children with Mb Down syndrome, and children undergoing circulatory arrest. **Results:** The serum concentrations of S-100 protein before the cardiac operation were found to be highest in neonates. Children with Down syndrome, regardless of age, had basal levels comparable to those in neonates. There was an increase in S-100 protein concentration immediately after extracorporeal circulation and a multivariate regression analysis showed this difference in S-100 protein concentration to be significant with respect to age ($p = 0.002$), perfusion time ($p < 0.001$), and circulatory arrest ($p < 0.001$), but the difference was not significant with respect to weight, Down syndrome, and core temperature ($p > 0.8$). In children younger than 1 month old and after circulatory arrest, levels of S-100 protein remained high at 5 hours after extracorporeal circulation. **Conclusion:** These findings emphasize the necessity of using age-matched reference values and taking perfusion time into consideration when S-100 protein levels are evaluated with respect to cerebral postperfusion injuries in pediatric patients undergoing cardiac operations. (J Thorac Cardiovasc Surg 1998;116:281-5)

A cerebral complication after a pediatric cardiac operation can be difficult to diagnose and to quantify, because clinical expressions are blunted by sedation and muscular relaxation. Continuous electroencephalography, transcranial Doppler ultrasonography, computed tomography, and magnetic resonance imaging

are methods that have been used to detect cerebral injuries, but these methods are expensive and time consuming. A specific biochemical marker for early detection of cerebral complications after pediatric cardiac operations would be of great value.^{1,2}

S-100 protein is a type of protein synthesized in astroglial cells in all parts of the central nervous system.³ Structural damage to the brain causes a selective leakage into the cerebrospinal fluid of S-100 protein. The aim of this study was to investigate the occurrence of S-100 protein in serum in pediatric patients undergoing cardiac operations with extracorporeal circulation (ECC).

Patients and materials

This study was approved by the ethical committee of the University of Lund.

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Fig. 1. Levels of S-100 protein before the operation in three age groups. Median is used as midpoint. *Upper box* value is the 75th percentile and *lower box* value is the 25th percentile. The *whiskers* represent the nonoutlier range. Outlier data points are represented by *open circles*. *** $p < 0.001$ between group 0 to 31 days ($n = 22$) and both group 1 to 12 months ($n = 20$) and group 1 to 16 years ($n = 48$). ### $p < 0.001$ between group 1 to 12 months and group 1 to 16 years.

During a 5-month period, we prospectively studied the appearance of S-100 protein in serum in 91 children of ages 0 to 16 years who were undergoing cardiac operations. In addition to these children and to enlarge the number of children in one group we did a follow-up study during the following 7 months in which we prospectively studied six children younger than 1 month old, and these children were included in the study.

Serum was analyzed for S-100 protein after the induction of anesthesia, immediately after the discontinuation of ECC, and 5 and 15 hours after ECC. Postoperative neurologic status was assessed daily by clinical examination.

The children were divided into three age groups and two separate groups: (A) age 0 to 1 month ($n = 22$); (B) age 1 to 12 months ($n = 20$); (C) age 1 to 16 years ($n = 48$); (D) all children with Down syndrome, ages 4 months to 6 years ($n = 7$); and (E) all children undergoing circulatory arrest procedures, ages 0 to 77 days, median 6 days ($n = 7$).

Premedication consisted of rectally given midazolam (0.5 mg/kg). Anesthesia was induced intravenously with atropine (0.02 mg/kg), fentanyl (5 μ g/kg), and thiopental (5 to 7 mg/kg). Intubation was performed with the use of pancuronium (0.2 mg/kg) relaxation. Anesthesia was maintained by a low dose (0.5% to 1%) of halothane or isoflurane and additional doses of fentanyl (5 μ g/kg) were given.

Cardiopulmonary bypass was performed with nonpulsatile perfusion during hypothermia (arterial blood from the oxygenator at 20° C) and when deep hypothermia and circulatory arrest were applied (arterial blood at 8° C, nadir temperatures of 15° to 20° C rectally and 12° to 15° C in hypopharynx). A membrane oxygenator (Cobe VPCML, Gambro, Lund, Sweden) and arterial line filters (Dideco D733 or D736, Täby, Sweden) were used. The circuit was primed with Ringer-acetate, mannitol, and Addex-THAM, and in children with a weight of less than 12 kg erythrocyte concentrate was added. The pump flow and mean arterial blood pressure were adjusted to the body surface and body temperature and if needed bolus injections of norepinephrine (0.1 to 1 μ g/kg) were given.

S-100 protein concentration was analyzed with use of a commercially available monoclonal two-site immunoradioactive assay, which detects the $\alpha\beta$ and $\beta\beta$ dimers (Sangtec 100, Sangtec Medical, Bromma, Sweden) as described by Westaby and associates.⁴ The blood samples were centrifuged to separate the serum, frozen to -20° C, and analyzed the next day. The lower sensitivity of the assay was 0.2 μ g/L.

Statistics. Computerized statistical analysis was performed with the SPSS for Windows statistical program (release 6.1). Data are presented as means plus or minus the standard deviations. Differences in S-100 protein levels between age groups were compared with unpaired Student's t tests and within groups A to E with Wilcoxon signed rank tests. A multivariate linear regression with differences in S-100 protein levels as dependent variables and with Down syndrome, weight, perfusion time, core temperature, circulatory arrest, and age (transformed by logarithm) as independent variables was tested. Correlation between differences in S-100 protein concentration and perfusion time, hematocrit, nadir core temperature, nadir perfusion pressure, and circulatory arrest time was calculated by linear regression analysis.

Results

S-100 protein in serum was detected in all neonates and some of the older children up to age 8 years immediately after induction of anesthesia but before the operation. S-100 protein levels were found to be highest in neonates, lower at ages between 1 month and 1 year, and lowest at ages older than 1 year (Fig. 1). Children with Down syndrome, regardless of age, had levels comparable to those in neonates (Fig. 2). The serum concentration of S-100 protein increased after ECC, but did not reach statistical significance in group D (Fig. 2).

The multivariate regression showed that weight, Down syndrome, and core temperature were all nonsignificant ($p > 0.80$) related to the increase in S-100 protein levels immediately after ECC, whereas age ($p = 0.002$), perfusion time ($p < 0.001$), and circulatory arrest ($p < 0.001$) were significant. With these three significant variables we were able

Fig. 2. Concentrations of S-100 protein in serum in groups A through E before ECC, immediately after ECC, and 5 hours and 15 hours after ECC (means and standard deviations are shown).

to explain 52% of the variation in S-100 protein level ($R^2 = 0.52$).

The linear regression analysis showed a correlation between the difference in S-100 protein level and perfusion time ($r = 0.58, p < 0.001$) (Fig. 3) and nadir core temperature during ECC ($r = 0.38, p < 0.001$). Circulatory arrest was shown in the multivariate regression to be a strongly significant variable related to the difference in S-100 protein levels. Although the number of observations was small ($n = 7$) a linear regression performed with the expectation that the S-100 protein level would increase after the arrest showed a slight significance ($r = 0.70, p = 0.042$). There was no correlation between the increase in S-100 protein level and hematocrit or nadir perfusion pressure during ECC.

In groups A and E the highest levels of S-100 protein appeared immediately after the discontinuation of ECC and 5 hours after ECC. In groups B and C the highest levels of S-100 protein appeared immediately after ECC followed by a decrease 5 hours after ECC (Fig. 2). Children with Mb Down syndrome, all of whom were older than 1 month, showed levels after ECC comparable to levels in neonates, but the change in the concentration of S-100 protein after ECC was not significant versus the concentration measured before ECC. The highest concentrations of S-100 protein appeared in group E. The single highest value, $15 \mu\text{g/L}$, obtained 5 hours after ECC, was noticed in a 3-day-old child in whom epileptic seizures developed 24 hours after ECC with hypothermic circulatory arrest. No clinical neurologic deficit was noticed when sedation was discontinued and the child was awake and extubated 2 days later.

Fig. 3. Correlation between the increase in S-100 protein level immediately after ECC and perfusion time ($n = 80, r = 0.58, p < 0.001$): 95% confidence limits are indicated.

Discussion

This study shows that measurable blood levels of astroglial S-100 protein are present in neonates and in some younger children before ECC. In children older than age 8 years there were no detectable levels of S-100 protein. The mean concentration of S-100 protein was highest in neonates and decreased with age. This is in contrast to findings in cerebrospinal fluid in which S-100 protein levels increased with age from 0.7 to 66 years.⁵ The concentration of S-100 protein in blood has always been below the limit of detection in adult patients before sternotomy.^{4,6,7} This finding of detectable S-100 levels before ECC in children shows that elevated concentrations of S-100 protein can appear in serum without obvious cerebral injury. A less selective permeability of the blood-brain barrier combined with a higher protein turnover in the neuron cells because of a fast maturation of the central nervous system in children may contribute to this. A low renal excretion may also contribute to an increased concentration of S-100 protein in serum in the youngest children. However, these findings emphasize that a moderately increased concentration of S-100 protein in adult patients must be interpreted carefully and may be caused by a reversible injury to the endothelial cells of the blood-brain barrier or a decrease in renal function during and after ECC.

The finding of an increased concentration of S-100 protein in children with Down syndrome may be explained by the duplication of chromosome 21, perhaps in conjunction with a more permeable blood-brain barrier. The S-100 protein gene is located on chromosome 1q21.³

Fig. 4. Correlation between the increase in S-100 protein level immediately after ECC and circulatory arrest time ($n = 7$, $r = 0.70$, $p = 0.042$): 95% confidence limits are indicated.

Our results show a significant correlation between the increase in S-100 protein levels and duration of ECC, which is in agreement with the findings of Westaby and associates⁴ in adult patients. We also found a correlation between the increase in S-100 protein level and the nadir core temperature during ECC. Kumar and associates⁷ suspected that the nadir core temperature in their patients might have influenced the S-100 protein release. They found higher S-100 protein levels in patients who were operated on at lower temperatures. However, children with a long perfusion time tend to have a lower nadir core temperature during ECC and our multivariate regression indicated that nadir core temperature was merely covariate with perfusion time and was not a significant cause of the increase in S-100 protein concentrations immediately after ECC. The relation between the increase in S-100 protein level immediately after ECC and circulatory arrest is interesting and more observations might help to better define the correlation between difference in S-100 protein level and circulatory arrest time.

It has been suggested that the concentration of S-100 protein in cerebrospinal fluid may be used to estimate the size of an ischemic brain injury,⁸ and it has also been suggested that the release of S-100 protein during ECC may be associated with a flow-related microembolization to the brain.^{4,9} We did not notice any clinical signs of postoperative neurologic injury, except in one child with transient epileptic seizures. More extensive neurophysiologic tests were not applied.

Our highest levels of S-100 protein appeared immediately after ECC followed by a decline, except after circulatory arrest and in neonates, in which

cases the decline was not detected until 15 hours after ECC. Other researchers have detected the highest levels of S-100 protein after ECC followed by a continuous decline.^{4,6} In a situation with extensive microembolization to the brain it seems more likely that a release of S-100 protein from unperfused brain areas would be postponed until the cellular breakdown appears. A more plausible explanation for the initial increase in S-100 protein level is therefore an increased permeability of the blood-brain barrier. The systemic inflammatory response syndrome and endothelial cell activation and injury are well known after cardiac operations.^{10, 11} The endothelium-leukocyte interaction with release of inflammatory mediators leads to damage of endothelial integrity, sticking of leukocytes in the microcapillary bed, and microcirculatory dysfunction. The increase in endothelial permeability could explain the augmented release of S-100 protein to the blood. This is in agreement with the finding of cerebral edema in normal patients after cardiopulmonary bypass investigated by magnetic resonance imaging.¹² The use of a bioactive heparin-coated surface in the cardiopulmonary bypass circuit, which attenuates the cellular and humoral response to ECC, also decreases the release of S-100 protein after ECC in adults and improves cerebral protection.¹³ The endothelial activation seems to be more extensive in less mature children and in children after circulatory arrest, judged by the peak levels of S-100 protein in our study. The use of a bioactive heparin-coated surface during ECC in these children might therefore be beneficial. Because the biologic half-life of S-100 protein has been estimated to be about 2 hours, the delay in elimination in neonates and after circulatory arrest could indicate a continuous release of S-100 protein from the brain. However, a decrease in glomerular filtration rate can also contribute to this.

In conclusion, we have found increased postperfusion levels of S-100 protein in children, with the highest values seen after circulatory arrest procedures. For further investigation of S-100 protein as a marker for cerebral injury, age and perfusion time have to be taken into consideration.

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Commentary

Overt cerebral injury occurs in a small percentage of patients after cardiac surgery and presents little problem in diagnosis. One the other hand, there is no easy method of detecting the subtle cerebral injury, evidenced by dysfunction in higher cognitive domains, which occurs in two thirds of patients soon after surgery and which persists in up to one third. In addition to the practical limitations of electroencephalography, computed tomography, magnetic resonance imaging, and transcranial Doppler ultrasonography, interpretation of abnormalities and their functional significance can be difficult. Neuropsychologic testing reproducibly detects cognitive dysfunction but is laborious and time consuming for both patients and examiners (limitations that are exaggerated or insurmountable in the pediatric population). The availability of a serum marker of cerebral injury would therefore be of considerable value.

S-100 protein represents a group of astroglial proteins widely distributed in the central nervous system. After head injury or cerebrovascular accident, S-100 protein leaks from structurally damaged cells into cerebrospinal

fluid and then blood, where its serum levels give some indication of the magnitude of structural injury. Over the past few years, a number of investigators have documented minor and transient elevations in serum S-100 protein levels after cardiac surgery and assumed, in the absence of overt cerebral damage, that this represents subtle cerebral injury.

The current study by Lindberg and colleagues addresses the important question of cerebral injury after pediatric surgery¹ and describes serial alterations in S-100 protein levels. While confirming a pattern of postoperative release broadly similar to that in adults, it provides further important observations regarding the kinetics of S-100 protein in children. Unlike the situation in adults, S-100 protein is detectable in the preoperative period in younger children and those with Down's syndrome. In an elegant analysis of their data, Lindberg and colleagues report that postoperative elevations in S-100 protein level correlate with younger age, increasing duration of perfusion, and the use of circulatory arrest. The title of the manuscript teasingly describes S-100 protein as a possible new marker for postperfusion cerebral injury, and this is, indeed, a tantalizing prospect. But is it likely?

Although large and prolonged increases in S-100 protein after cardiac surgery (in the absence of renal failure) have been correlated with structural brain injury, the functional significance of the more commonly observed pattern of early, modest, and temporary elevations in S-100 protein soon after cardiopulmonary bypass is uncertain. This may simply represent a cellular "washout" phenomenon accompanied by a temporary increase in the permeability of the blood-brain barrier as opposed to true neuronal injury. If so, the value of S-100 as a marker of subtle cerebral injury is reduced.

Interpretation of the significance of modest postoperative elevations in S-100 protein is compounded in the pediatric population because of its detectability in the preoperative period, its variability with age, conflicting evidence over the effects of hypothermia on its release, and the need for total circulatory arrest. Furthermore, as my colleagues and I² have previously reported, intracardiac surgery itself results in larger postoperative elevations in S-100 protein.

Lindberg and colleagues are to be congratulated on an excellent observational study. However, until there is substantial evidence correlating modest postoperative elevations in S-100 protein levels with structural and/or functional neuronal injury, the clinical significance of such findings must be interpreted cautiously.

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