Cytokine and S100B levels in paediatric patients undergoing corrective cardiac surgery with or without total circulatory arrest

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Received 23 September 1998; received in revised form 29 March 1999; accepted 7 April 1999

Abstract

Objectives: Neurological damage following cardiopulmonary bypass (CPB) is difficult to objectively evaluate in infants. In adults, serum elevations of astroglial S100B correlate with proven brain injury independent of operative temperature. The deleterious effects of inflammatory cytokines, generated during CPB, on the brain have not been studied in infants using S100B as a marker for cerebral injury.

Methods: Twelve neonates, weighing 3.3 ± 0.2 kg (total circulatory arrest group (TCA)) and 12 infants weighing 7.0 ± 1.0 kg (cardiopulmonary bypass group (CPB)) underwent corrective cardiac surgery for various pathologies. Serial blood samples on induction, at the end of CPB, 30 min, 2 h and 24 h after the administration of protamine, were taken. The resultant plasma was frozen to \(-80^\circ\text{C}\) and stored for batch analysis. Cytokines were measured using ELISAs and S100B using a luminometric assay.

Results: The TCA group were younger and experienced a longer perfusion time than the CPB group (137 ± 8 vs. 113 ± 7, \(P = 0.04\)). The mean TCA time was 23 ± 4 min. The TCA group had significantly higher levels of IL-6 (\(P = 0.001\)), IL-8 (\(P = 0.005\)) and S100B (\(P = 0.002\)) at 24 h. C5b-9 levels were significantly lower in the TCA group: end of CPB (\(P = 0.001\)), 30 min (\(P < 0.001\)), 2 h (\(P = 0.002\)). There was a weak, but significant correlation between IL-6 levels at the end of CPB and S100B levels 2 h later (\(r = 0.55, P = 0.03\)). Long extubation times were associated with high 24-h S100B levels (\(r = 0.52, P = 0.01\)).

Conclusions: (1) The TCA group have prolonged rises of IL-6, IL-8 and S100B. (2) The TCA group generates significantly lower complement. (3) Astroglial injury, seen after surgery, may, in part, be cytokine mediated. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Cerebral injury; S100B; Cytokines; Inflammation

1. Introduction

Cerebral injury continues to be a major complication after corrective cardiac surgery [1]. Recent evidence attributes the pathophysiology to macro- and microemboli generated during cardiopulmonary bypass (CPB) [2]. In addition, periods of total circulatory arrest (TCA) have been reported to confound the problem [3]. Although neuropsychometric testing is used in adult patients to detect subtle degrees of CNS injury [4], this option is not suitably developed for the paediatric population. Therefore serum markers for cerebral damage would be of paramount importance.

S100B is an astrocyte specific protein whose serum concentration rises after cerebral damage [5]. After cardiac surgery, a characteristic temporal change in levels has been reported [3,6,7]. These serum rises have been correlated with neuropsychological deficit [8]. Greater rises have been reported in patients who required TCA during surgery [3].

The systemic inflammatory response syndrome (SIRS) is incriminated in organ damage following CPB [9]. The proposed mechanism involves activated platelets, monocytes and polymorphonucleocytes releasing vasoactive substances and cytokines, which in turn alter capillary permeability and cause direct local cellular injury [10–13].

The aim of this study was to explore the relationship between serum cytokine rises and the degree of astroglial damage incurred in infants undergoing corrective cardiac surgery with or without TCA.

2. Methods

Local ethical approval and informed consent was obtained for this study.
2.1. Patients

The demographic data is summarized in Table 1. This shows significant differences between the groups (CPB (n = 12) and TCA (n = 12)) with respect to weight and duration of CPB. The surgical procedures performed are outlined in Table 2. The CPB patients underwent surgery for tetralogy of Fallot (n = 7) and ventricular septal defects (n = 5). In the TCA group, surgery for transposition of the great arteries (n = 9) was the predominant operation.

2.2. Anaesthesia and cardiopulmonary bypass

All patients received a similar balanced anaesthetic, including nitrous oxide, a neuromuscular blocking agent and opiate analgesia. Heparin (300 IU/kg) was administered prior to aortic cannulation and the activated clotting time (ACT) was maintained above 450 s. The bypass circuit consisted of a non-pulsatile roller pump (Stocker Instrumente GmbH, Munich, Germany), a membrane oxygenator (VCPL; Cobe Laboratories Inc, Lakewood, USA), a hard shell venous reservoir and polyvinyl tubing. The priming solution (containing 2000 IU heparin) was an admixture of whole blood and Hartmann’s solution 2.2. Anaesthesia and cardiopulmonary bypass

2.3. Postoperative care

In the intensive care unit, midazolam and morphine sulphate were given to maintain sedation and analgesia. Monitoring of haemodynamic parameters (heart rate and rhythm, systemic arterial, central venous and left atrial pressures) and measurement of urine output was continued as necessary. Appropriate inotropes were used to maintain haemodynamic parameters.

2.4. Blood samples

Blood samples were drawn from the central venous line after induction of anaesthesia, at the end of CPB, 30 min, 2 h and 24 h after the administration of protamine. The 1st ml aspirated was not used for analysis, it was returned to the patient after the sample for evaluation was withdrawn. The samples for analysis were inserted into ethylenediaminetetraacetic acid (EDTA) tubes for immediate assessment of haematocrit value, platelet count and leucocyte count and into sodium citrate tubes for all other analyses. The citrated blood was centrifuged for 10 min at 3000 rev./min. The resultant plasma was separated, aliquoted and stored for batch analysis at −80°C.

2.5. Cytokine analysis

Enzyme-linked immunoassays (ELISA) for IL-6 and IL-8 were performed using commercial kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. In brief, samples were diluted in an equal volume of diluent and incubated in wells coated with monoclonal anti-cytokine antibody. After washing six times, they were incubated with a polyclonal anti-cytokine antibody conjugated to horseradish peroxidase. After further washing, substrate solution containing tetramethylbenzidine and hydrogen peroxide was added, the reaction was stopped after 20 min with sulphuric acid (2 mol/l). The absorbances were measured using a microplate reader set at 450 nm with wavelength correction set to 570 nm. All assays were performed in duplicate. The intra and inter assay coefficients of variation were less than 5%. The limit of sensitivity for the assays were: IL-6 (3 pg/ml), IL-8 (20 pg/ml) and C5b-9 (16 ng/ml).

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No TCA (n = 12)</th>
<th>TCA (n = 12)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>7 ± 1</td>
<td>3.3 ± 0.2</td>
<td>0.003</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>113 ± 7</td>
<td>137 ± 8</td>
<td>0.04</td>
</tr>
<tr>
<td>Cross-clamp time (min)</td>
<td>66 ± 4</td>
<td>62 ± 4</td>
<td>0.53</td>
</tr>
<tr>
<td>Extubation time (h)</td>
<td>17 ± 5.5</td>
<td>27 ± 5</td>
<td>0.20</td>
</tr>
<tr>
<td>ITU time (h)</td>
<td>54 ± 8</td>
<td>33 ± 11</td>
<td>0.14</td>
</tr>
<tr>
<td>TCA time (min)</td>
<td>–</td>
<td>23 ± 4</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Pathology</th>
<th>TCA group (n = 12)</th>
<th>CPB group (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGA</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>TOF</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>VSD and aortic interruption</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>VSD</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>Truncus</td>
<td>1</td>
<td>–</td>
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</tbody>
</table>
2.6. S100B assay

Plasma S100B levels were measured using a monoclonal luminometric assay (LIA), (Sangtec® 100 LIA, AB Sangtec Medical, Bromma, Sweden), both using three monoclonal antibodies SMST 12, SMSK 25 and SMSK 28 to detect the B chains in the S100BB and S100A1B. Aliquots of 100 μl plasma samples or the standards were diluted with 100 μl of bovine serum albumin (BSA) in test tubes provided in the kit. These tubes were already coated with anti-S100B antibody. After incubating for 1 h, the tube was washed three times with wash buffer and 200 μl of a luminescence labelled antibody (tracer) was added. After a further 2 h incubation, during which this antibody was captured by anti-S100B antibody on the test tube surface, the unbound tracer was washed out, and the residual antibody was measured using a luminometer. This automatically computed against the standard curve by recording the total luminescence for each given standard provided in the kit. All assays were performed in duplicate. The intra assay coefficients of variation were less than 5%. The limit of sensitivity of the assay was 0.02 ng/ml.

2.7. Statistical analysis

SPSS for Windows (version 7.0) was used for data analysis. Patient characteristics are expressed as means and standard error of the mean and plasma concentrations are represented as medians and interquartile ranges. Differences between the patient groups were assessed using a Mann–Whitney test. Spearman’s correlation coefficient was used to determine the relationship between defined parameters and measurements. The box-plot and whisker graphs represent the individual data points (circles) and the best fit line of linear regression (straight dotted line).

3. Results

There were no deaths or evidence of stroke in this series. All S100B, cytokine and complement concentrations were significantly raised compared to the preoperative values, irrespective of whether patients received TCA or not. The temporal release of S100B is illustrated in Fig. 1. Peak levels were observed at the termination of CPB in both groups. The late postoperative S100B concentrations were significantly higher in the TCA group: at 2 h $P = 0.007$; at 24 h $P = 0.005$ (Mann–Whitney).

The plasma profiles of the inflammatory markers are shown in Figs. 2–4. The TCA patients have significantly higher IL-6 and IL-8 concentrations 24 h after surgery $P = 0.001$ and $P = 0.002$, respectively (Mann–Whitney). With complement, however, significant differences were observed immediately after the cessation of CPB, 30 min after administering protamine and at 2 h ($P = 0.001$, $P < 0.001$, $P = 0.002$ (Mann–Whitney)).

The temporal release of cytokines IL-6 and IL-8 are similar to that of S100B. The 2-h S100B levels correlate with the end of CPB IL-6 concentrations ($r = 0.55$, $P = 0.03$ (Spearman’s Rank correlation)), this is illustrated in Fig. 5. Whereas, there is a significant correlation between the 24 concentrations of S100B and IL-8 ($r = 0.77$, $P = 0.002$). In the CPB group, the duration of perfusion, correlated weakly, but significantly, with peak IL-6 concentrations ($r = 0.66$, $P = 0.018$).

A relationship between prolonged intubation and raised 24-h-S100B concentrations was also observed ($r = 0.52$, $P = 0.01$). One neonate was ventilated for 5 days, this patient’s 24-h-S100B concentration was 2.68 ng/ml.

![Fig. 1. A box-plot and whisker graph showing the plasma profiles of S100B in all patients. The graphs illustrate the median (bold black line), the upper and lower quartiles (the box), the range of data excluding outliers (the whiskers), outliers (circles) and extreme outliers (asterisks).](https://academic.oup.com/ejcts/article-abstract/16/1/32/453411)

![Fig. 2. A box-plot and whisker graph showing the plasma profiles of IL-6 in all patients. The graphs illustrate the median (bold black line), the upper and lower quartiles (the box), the range of data excluding outliers (the whiskers), outliers (circles) and extreme outliers (asterisks).](https://academic.oup.com/ejcts/article-abstract/16/1/32/453411)
4. Discussion

At present there are no tools to objectively evaluate early cerebral injury following cardiac surgery in paediatric patients. The pathophysiology of neuropsychologic injury is multifactorial. Recent reports suggest that an important effect is derived from microemboli [2] and hypoperfusion, which may be local or regional [14]. Our data suggests that the aetiology may be more complex than originally thought. It is possible that inflammatory cytokines may play a role.

The temporal changes in IL-6 have corroborated with previous studies [15,16]. IL-6 rose significantly after the end of CPB and continued to rise for 2 h. There was an observed relationship between the end of CPB concentration of IL-6 and S100B 2 h after surgery. This 2 h delay in S100B elevation may implicate this inflammatory mediator as a potential astroglial toxin. The possibility of IL-6 merely reflecting intra-operative astroglial damage is unlikely, due to the temporal relationship of IL-6 with S100B. IL-6 has also been reported to be involved in the acute phase response and it may continue to be released from other cells after incurring astroglial damage; and indeed plasma IL-6 levels correlate with postoperative complications in patients undergoing major surgery [17]. This raises the question of whether IL-6 causes direct astroglial injury or whether its action is interlinked with other cytokines. IL-1 is not only cytotoxic, it also stimulates IL-6 production [18]. However, there have been difficulties in measuring IL-1 in plasma. This has focused interest on measuring its specific receptor antagonist (IL-1ra) [18,19]. IL-1ra rises in humans when intravenous IL-1 is administered [18]. Support for IL-6 being directly injurious to astrocytes is derived from in vitro data which shows a significant release of S100B from astrocytes into the supernatant, when IL-6 is added to the incubating medium of normal human astrocytes grown in tissue culture flasks (unpublished data, Bhattacharya, K., Perera, T.S.P.). Also, data from murine neurone and glial cell cultures have reported that cytokines, including IL-1 and IL-6, induced dramatic neuronal cell death [20]. This evidence is suggestive of direct toxicity, however, the role of IL-1 needs to be further evaluated.

IL-8, which is predominantly produced by macrophages, fibroblasts, neutrophils and endothelial cells, has been reported as a potent activator of neutrophil chemotaxis [12] and as a priming agonist for neutrophils (increasing the percentage of cells generating an oxidative burst during subsequent stimulation) [21]. Recently, the deleterious pulmonary effects of IL-8 have been curtailed in rabbits using monoclonal IL-8 antibody [22]. In our study, there was a weak, but significant, correlation between prolonged intubation and 24 h IL-8 concentrations ($r = 0.52$, $P = 0.01$). This implies that a persistent elevation of IL-8 may prolong or be a result of lung injury sustained during the procedure. This relationship has also been observed in adult patients undergoing CABG [19].

Fig. 3. A box-plot and whisker graph showing the plasma profiles of Cb5 in all patients. The graphs illustrate the median (bold black line), the upper and lower quartiles (the box), the range of data excluding outliers (the whiskers), outliers (circles) and extreme outliers (asterisks).

Fig. 4. A box-plot and whisker graph showing the plasma profiles of IL-8 in all patients. The graphs illustrate the median (bold black line), the upper and lower quartiles (the box), the range of data excluding outliers (the whiskers), outliers (circles) and extreme outliers (asterisks).

Fig. 5. A scattergraph showing the relationship between peak IL-6 concentrations and 2-h-S100B levels. The graph shows the individual data points (circles), best fit line of linear regression (straight dotted line).
The TCA patients exhibited significantly higher postoperative concentrations of S100B than the CPB group. This corroborates findings of other authors who used different end points to assess the adverse neurological effects of TCA in paediatric patients [22–25]. The temporal release pattern is similar to adults, except that the preoperative and the late postoperative concentrations are significantly higher [26]. This may be attributed to an immature blood-brain barrier and a higher astroglial turnover in the neonates [26]. In our study, this difference was also observed. The TCA patients were younger than the CPB patients and their preoperative values were significantly higher \( (P = 0.002) \) (Mann–Whitney)). There was no direct relationship observed between the duration of TCA and postoperative S100B levels, although this is reported in adult patients [3]. This may be explained by the cumulative effects of emboli, haemodilution, and SIRS, without the protection of a functional blood-brain barrier, inducing sufficient damage to offset the local and regional effects of hypoperfusion secondary to TCA. Furthermore, deep hypothermia has been observed to improve cerebral oxygenation in piglets [24]. Our study also demonstrated a relationship between late S100B levels and prolonged extubation time \( (r = 0.52, P = 0.01) \) (Spearman’s)). This implies that patients with greater astroglial injury are slower to wean from a ventilator.

Complement activation during CPB precedes that of IL-6 [10]. This is consistent with our findings. The levels of C5b-9 were significantly lower at all time points, except at 24 h in the TCA group: \( (P = 0.28) \); and \( P = 0.001, 30 \text{ min}, P < 0.001; 2 \text{ h}, P = 0.002 \) (Mann–Whitney)). These low levels may be due to immature activity of the complement pathways and partial protection offered by TCA [15].

This study was limited by the numbers of patients analysed, the sensitivities of the assays used and the complex interactions between the inflammatory cytokines. Clearly, there is a need to investigate this further, and as some proinflammatory cytokines stimulate and upregulate each other’s activity, it may be worthwhile measuring an anti-inflammatory cytokine such as IL-1ra which may reflect the cumulative effect of the proinflammatory cytokines [19].

Our objective was to evaluate a potential role for inflammatory mediators such as IL-6 and IL-8 in the cerebral damage observed during, and after, corrective cardiac surgery in paediatric patients. We conclude that our data supports a relationship between the two, although the influence of IL-6 and IL-8 may de different temporally and mechanistically, and may also recruit other cytokines to induce cellular injury.

Acknowledgements

The authors would like to thank: Raj Mattu, University of Warwick; Ramesh Patel, Walsgrave Hospital, for their help in preparing the manuscript; Andrew Vale, University of Leeds; Dr. Mario Cortina-Borja, University of Oxford, for statistical consultation; Lena Nyberg, AB Sangtec Medical, for help and advice with the S100B assays.

References

Appendix A. Conference discussion

Dr R. Jonas (Boston, MA, USA): I would take issue with your statement at the beginning that it is not possible to undertake developmental testing of infants. The Bayley scale is specifically designed for developmental testing of children as young as, I believe, about 6 months? And certainly by 12 months the Bayley scale is appropriate. My question relates to baseline differences related to age and S100B levels. Others have previously reported that neonates and young infants have a higher baseline level of S100B relative to adults, for example. Your own data, I noticed, showed that there was a higher level in the neonatal group, which was the circulatory arrest group, relative to the continuous bypass group, who were older children. Is there a statistically significant difference in the percent change from baseline for the circulatory arrest patients relative to the other patients, and at what time points is that difference significant?

Mr Bhattacharya: There is a significant difference in the S100 values between the TCA patients and the non-TCA patients. The mean weight for the TCA group was 3 kg and for the non-TCA group was 7 kg. There is a significant difference between the changes in S100 at 2 h in our study. The levels in the TCA patients preoperatively and at 24 h were similar. I do take your point that the levels are higher. However, if you think about the weight of the child and the sort of amount of brain tissue involved, the changes may also be determined by that. So you’ve got a small child with a high level initially and you’ve got a heavier child who releases less S100, so just by that we thought maybe there is more cerebral injury being incurred in the TCA group.