Adrenomedullin Blood Concentrations in Infants Subjected to Cardiopulmonary Bypass: Correlation with Monitoring Parameters and Prediction of Poor Neurological Outcome

Pasquale Florio,1 Raul Abella,2 Emanuela Marinoni,3 Romolo Di Iorio,3 Claudio Letizia,3 Marco Meli,4 Teresa de la Torre,2 Felice Petraglia,1 Alessandro Cazzaniga,2 Alessandro Giamberti,2 Alessandro Frigiola,2 and Diego Gazzolo5,6

1 Department of Pediatrics, Obstetrics, and Reproductive Medicine, University of Siena, Siena, Italy; 2 Department of Cardiac Surgery, S. Donato Milanese University Hospital, S. Donato Milanese, Italy; 3 Laboratory of Perinatal Medicine and Molecular Biology and Department of Internal Medicine, University “La Sapienza”, Rome, Italy; 4 Department of Pediatric Intensive Care, Hesperia Hospital, Modena, Italy; 5 Department of Pediatrics and Neuroscience, G. Gaslini Children’s Hospital, University of Genoa, Genoa, Italy; and 6 Department of Fetal, Maternal, and Neonatal Health, G. Garibaldi Hospital, Catania, Italy; *address correspondence to this author at: Department of Maternal Fetal and Neonatal Health, G. Garibaldi Hospital, Via Palermo 636, I-95100 Catania, Italy. e-mail dgazzolo@hotmail.com.

BACKGROUND: Brain injury is a major adverse event after cardiac surgery, especially when extracorporeal circuits are used. We evaluated whether cardiopulmonary bypass (CPB) affects cerebrovascular resistance and plasma concentrations of adrenomedullin (AM), a vasoactive peptide regulating cerebral blood flow.

METHODS: We evaluated 50 infants (age <1 year) with congenital heart defects, matched according to a 2-year follow-up; 40 infants had no overt neurological injury, and 10 had brain damage. Blood samples were taken before surgery, during surgery before CPB, at the end of CPB, at the end of surgery, and at 12 h after surgery. Neurological outcome was evaluated before surgery, during surgery before CPB, at the end of CPB, and after surgery. As single markers for predicting neurological abnormalities, AM (cutoff: 17.4 ng/L) achieved a sensitivity of 100% and a specificity of 73.0% and MCA PI (cutoff value: 1.8) a sensitivity of 100% and a specificity of 56.8%.

RESULTS: The highest MCA PI values and lowest AM concentrations occurred at the end of CPB and of the surgical procedure. Infants who developed abnormal neurologic sequelae had significantly (P < 0.001 for both) higher MCA PI values and lower AM concentrations than patients with normal neurologic outcome at the end of CPB and after surgery. As single markers for predicting neurological abnormalities, AM (cutoff: 17.4 ng/L) achieved a sensitivity of 100% and a specificity of 73.0% and MCA PI (cutoff value: 1.8) a sensitivity of 100% and a specificity of 56.8%.

CONCLUSIONS: AM concentrations and MCA PI patterns change during CPB, mainly in infants with brain damage, and may be useful for early identification of infants at risk for brain damage.

Pedicic open-heart surgery performed with cardiopulmonary bypass (CPB) has harmful cerebrovascular effects because intra- and postoperative hypoperfusion, post-CPB reperfusion, and embolization and/or thermal injury deeply affect the cerebrovascular autoregulation system (1). We investigated whether open heart surgery with CPB changes cerebrovascular resistance and adrenomedullin (AM) secretion, and whether AM measurement is useful for monitoring cerebral distress during CPB. AM, a 6-kD vasoactive peptide (2), affects cerebral circulation and vasodilates cerebral arterioles without modifying systemic blood pressure or other cardiovascular variables (3). AM plays a role in fetal cerebral hemodynamic modifications due to hypoxia (4), and in preterm newborns who develop intraventricular hemorrhage, an early increase of AM is related to a series of events causing loss of the cerebral vascular autoregulation system, leading to cerebral bleeding due to reperfusion (5).

From March 2002 to September 2004, we conducted a case-control study of 50 infants (24 males, 26 females) without preexisting neurological disorders or other comorbidities, admitted to our referral centers for cardiac diseases including Fallot tetralogy (n = 35), great-artery transposition (n = 7), aortic stenosis (n = 4), and tricuspid atresia (n = 4). Informed consent from parents or guardians was obtained before patient inclusion in the study, which was approved by the local human-investigation committee. The group without brain injury included 40 infants with no overt postoperative neurological injury; the brain-damage group included the infants (n = 10) who developed neurological injury identified during a 2-year follow-up period. Heparinized blood samples were taken at 5 time-points [before surgery (time 0), during surgery before CPB (time 1), at the end of CPB (time 2), at the end of surgery (time 3), and 12 h after surgery (time 4)] and assayed for AM. Standard monitoring variables and laboratory values were also recorded.

Anesthetic and standardized CPB (α-stat regimen) techniques were performed according to previously published protocols (6). Neurological development was assessed in all participants by a single examiner.
(D.G.) with the Amiel-Tison test (7) preoperatively, on postoperative day 7, and 2 years after surgery. Results were compared for infants of the same age (in months) and scored as “normal” or “abnormal”. A pulsed-Doppler apparatus (Acuson 128SP5) was used for blood flow velocity measurement of the middle cerebral artery pulsatility index (MCA PI), performed at the same time that blood was drawn. AM was measured by a commercial RIA (Phoenix Pharmaceuticals). After extraction and purification (5), the analytical detection limit was 2.5 ng/L, intra- and interassay CVs were 5.3% (6 replicates) and 11.7% (9 replicates), respectively, for concentrations between 5 and 1280 ng/L (see supplemental data files at http://www.clinchem.org/content/vol54/issue1/). The assay does not react with calcitonin gene-related peptide, endothelin-1, /H9251-atrial natriuretic peptide, or brain natriuretic peptide (8).

The Kolmogorov–Smirnov test showed values to have a gaussian distribution, and data were expressed as the mean (SD). Statistical significance was assessed using 1-way ANOVA for repeated measures (followed by the post hoc Tukey test for multiple comparisons) and the unpaired t-test when only 2 groups were compared. Pearson correlation coefficients were calculated to test the linear correlation between AM concentrations and various clinical variables. By the ROC curve analysis (9), we estimated the probability of the development of neurological abnormalities according to the AM or MCA PI values and compared this probability with the pretest probability (the prevalence of brain damage in the entire group of infants). Statistical significance was assumed for $P < 0.05$.

Age, weight, male/female distribution, and CPB, clamping, and circulatory arrest durations did not differ between the 2 groups (Table 1). The length of stay in the pediatric intensive care unit was significantly higher ($P < 0.01$) in infants with brain damage at follow-up. Clinical laboratory values and standard monitoring variables recorded at the predetermined time points remained within the reference limits ($P > 0.05$; data not shown). Examination on day 7 after the surgical procedure showed neurological abnormalities in 10 infants (brain damage group: hypotonia-hypertonia syndrome, $n = 4$; seizures, $n = 2$; hemisindrome, $n = 4$), and in these infants neurological abnormalities were still present at the 2-year follow-up (hypotonia-hypertonia syndrome, $n = 6$; hemisindrome, $n = 4$). In both groups of children, MCA PI values showed an increasing trend throughout the time points evaluated, reaching the maximum peak at the end of CPB and of the surgical procedure (Fig. 1). In both groups MCA PI values were significantly ($P < 0.001$, for all) lower before surgery than at the end of CPB and of surgery (Fig. 1). However, before surgery and before CPB MCA PI, values were significantly ($P < 0.001$, for both) higher in children with brain damage.

### Table 1. General characteristics and CPB–monitoring variables for the 50 infants in the 2 study groups who underwent repair of congenital heart defects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No brain injury ($n = 40$)</th>
<th>Brain damage ($n = 10$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, days</td>
<td>126 (110)</td>
<td>131 (99)</td>
</tr>
<tr>
<td>Weight, g</td>
<td>5,234 (2,440)</td>
<td>5,311 (2,566)</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>20/21</td>
<td>4/5</td>
</tr>
<tr>
<td>Neurological examination results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before surgery, n</td>
<td>40/0</td>
<td>10/0</td>
</tr>
<tr>
<td>After surgery (day 7), n</td>
<td>40/0</td>
<td>0/10</td>
</tr>
<tr>
<td>After surgery (2 years), n</td>
<td>40/0</td>
<td>0/10</td>
</tr>
<tr>
<td>CPB prime volume, mL</td>
<td>380</td>
<td>380</td>
</tr>
<tr>
<td>CPB, min</td>
<td>106.6 (37)</td>
<td>101.9 (38)</td>
</tr>
<tr>
<td>CPB cooling time, min</td>
<td>12.9 (1.6)</td>
<td>12.8 (1.7)</td>
</tr>
<tr>
<td>DHCAc time, min</td>
<td>41.5 (7.2)</td>
<td>41.4 (7.7)</td>
</tr>
<tr>
<td>CPB rewarming time, min</td>
<td>20.8 (9.4)</td>
<td>21.0 (10.6)</td>
</tr>
<tr>
<td>Circulatory arrest time, min</td>
<td>39 (4)</td>
<td>41 (3)</td>
</tr>
<tr>
<td>Intensive care stay, days</td>
<td>11.6 (2.2)$^b$</td>
<td>18.2 (2.9)</td>
</tr>
</tbody>
</table>

*a* Data are expressed as mean (SD), *n*, or number of patients with normal/abnormal results in neurologic examinations.

$b$ $P < 0.01$.

$^c$ Deep hypothermic circulatory arrest.
Fig. 1. Middle cerebral artery pulsatility index (MCA PI) (upper panel) and plasma adrenomedullin concentrations (lower panel).

Values were measured at different time points (see text) in a group of infants who underwent surgery for congenital heart defects and had no overt neurological injury (n = 40; open circles) or poor neurological outcome with brain damage (n = 10; black circles) at follow-up. Horizontal bars represent the mean. MCA PI: *P < 0.001 vs time 2 and 3; adrenomedullin: *P < 0.001 vs time 1, 2, and 3.
than in those with normal neurological outcomes, and these values remained significantly ($P < 0.001$) higher in the brain damaged infants at 12 h after surgery (Fig. 1).

AM concentrations varied inversely with respect to MCA PI values; AM concentrations decreased significantly ($P < 0.001$) throughout the time points in both groups of children, with the minimum dip at the end of CPB (Fig. 1). Before surgery and CPB, AM did not differ between the 2 groups of children ($P > 0.05$, for both); AM also did not differ between the 2 groups at time 4 (at 12 h after surgery) ($P > 0.05$). At the end of CPB and of surgery, however, AM was significantly lower ($P < 0.001$, for both) in the brain damage group than in the healthy controls (Fig. 1). No significant correlations were found between AM and MCA PI values or between AM or MCA PI and CPB variables at the end of CPB (data not shown).

According to ROC curve analysis, the value of AM as a diagnostic test was higher at the end of CPB, because at the cutoff concentration of 17.4 ng/L AM achieved a sensitivity of 100% (95% CI: 69.0%–100%) and a specificity of 73.0% (95% CI: 55.9%–86.2%) as a single marker for predicting brain damage (area under the ROC curve: 0.897; 95% CI: 0.773–0.966), with positive and negative likelihood ratios (LR) of 3.7 and 0.0, respectively. The higher value of MCA PI as a diagnostic test was also observed at the end of CPB, because at the cutoff value of 1.8, MCA PI achieved a sensitivity of 100% (95% CI: 69.0%–100%) and a specificity of 56.8% (95% CI: 39.5%–72.9%) as single marker to predict the occurrence of neurological abnormalities (area under the ROC curve: 0.822; 95% CI: 0.682–0.918), with positive and negative LR of 2.31 and 0.0, respectively.

In the present study we found that infants subjected to CPB had decreased AM concentrations and increased cerebrovascular resistance during and after the surgical procedure. AM infusion in animals increases cerebral blood flow in a dose-dependent manner (3, 10), suppresses the reduction in regional cerebral blood flow, and prevents ischemic brain injury after middle cerebral artery occlusion (in vivo in rats) (10). In humans AM is involved in the regulation of fetal cerebral vascular hemodynamic mechanisms for adaptation to hypoxia (4) and participates in the ischemia-reperfusion mechanisms leading to intraventricular hemorrhage in preterm newborns (5). These findings and the evidence of lowest AM concentrations coinciding with high MCA PI values suggest that changes of AM in the bloodstream of infants who have undergone cardiac surgery with CPB may reflect cerebral distress due to extracorporeal circulation, arising as a specific response to operative stress and hemodynamic changes. The increase of MCA PI is suggestive of vasoconstriction, and it is reasonably associated with a decrease of AM plasma concentrations. Indeed, the AM decrease at time 1 was not due to priming solution, because at this time-point CPB was no longer being performed. On the contrary, the CPB procedure and cooling/rewarming phases may affect AM at different monitoring time-points, as suggested by the AM increase in fetuses with intrauterine growth restriction and newborns with reperfusion injury (4, 5). Conversely, when damage is mainly due to ischemia a finding of decreased AM can be reasonably expected.

Animal data support the hypothesis that microinjections of AM in the area postrema produce significant blood pressure and heart-rate changes, suggesting an involvement of AM in cardiovascular regulation (11–13). AM is measurable in plasma from adult patients undergoing open heart surgery with CPB, in whom concentrations in the internal jugular vein may reflect local cerebral synthesis and release of the peptide (12). The association of open heart surgery by means of CPB with brain injury (6, 14) along with our findings at follow-up of significantly lower AM concentrations in infants with neurological abnormalities than in those without suggest that deeper vascular insult during the surgical procedure leads to more profound decreases of AM, as also indicated by changes in MCA PI. We also evaluated the clinical usefulness of AM and MCA PI measurements to identify children at risk of poor neurological outcome, at a stage (time 2, after CPB) when injury may have already occurred, but before the appearance of neurological abnormalities. At cutoffs chosen by use of the ROC curve analysis, sensitivity and specificity for predicting neurological abnormalities at the end of CPB were 100% and 73.0% for AM and 100% and 56.8% for MCA PI.

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**References**


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