The dependence of myocardial damage on age and ischemic time in pediatric cardiac surgery

Tomomi Hasegawa, MD^{a,b}

Masahiro Yamaguchi, MD, PhD^b

Naoki Yoshimura, MD, PhD^b

Yutaka Okita, MD, PhD^a

Objective: Heart fatty acid-binding protein is a rapid indicator for assessment of myocardial damage in cardiac surgery. The purpose of this study was to investigate the effects of age and ischemic time on the biochemical evidence and clinical outcomes of myocardial damage in pediatric cardiac surgery.

Methods: A prospective observational cohort study conducted over 2.5 years was performed in 98 consecutive patients (51 infants and 47 children) undergoing cardiac surgery for ventricular septal defects. Serial measurements of serum levels of heart fatty acid-binding protein and the respective areas under the curve were obtained, with particular reference to age and aortic crossclamp time. Assessment of clinical outcomes included inotropic support, ventilatory support, and intensive care unit stay.

Results: There was a linear dependence of the logarithm of age and the logarithm of heart fatty acid-binding protein release ($r = 0.737$, $P < .0001$). This logarithm-logarithm plot showed a power function of age for heart fatty acid-binding protein release. The exponent and amplitude parameter of the power function was the aortic crossclamp time. Compared with children, infants had significantly more myocardial damage and worse clinical outcomes, and these factors were related to the aortic crossclamp time.

Conclusions: The younger the age of patients, the more vulnerable are their myocardia to injury caused by ischemia during definitive repair of congenital heart disease. Therefore, perioperative management for pediatric patients after cardiac surgery should be performed, taking into consideration the dependence of the myocardial damage on age and ischemic time.

From the Division of Cardiovascular, Thoracic, and Pediatric Surgery, Department of Cardio-pulmonary and Vascular Medicine,^a Kobe University Graduate School of Medicine, and the Department of Cardiothoracic Surgery,^b Kobe Children's Hospital, Kobe, Japan.

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Address for reprints: Yutaka Okita, MD, Division of Cardiovascular, Thoracic, and Pediatric Surgery, Department of Cardio-pulmonary and Vascular Medicine, Kobe University Graduate School of Medicine, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan (E-mail: yokita@med.kobe-u.ac.jp).

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Because of recent advances in surgical technique, myocardial preservation and postoperative care have resulted in complete repair of many congenital heart defects in the neonatal period or early infancy. On the other hand, several investigators have reported that immature myocardium in the pediatric heart is more vulnerable to injury than mature myocardium in the adult heart.¹⁻³ Perioperative myocardial injury is a major determinant of cardiac dysfunction after operations for congenital heart disease. Therefore, it is very important to detect and to evaluate the degree of myocardial injury as soon as possible after the operative procedure. Previously, we reported that heart fatty acid-binding protein (HFABP) is a rapid marker for assessment of myocardial damage and clinical outcome in pediatric cardiac surgery.⁴ HFABP is a small intracellular protein consisting of 132 amino acid residues and weighing 14.9 kd. It is hydrophilous and abundant in the cytoplasm of cardiomyocytes in its free form. Its physiologic role is the transport of long-chain fatty acids from the cell membrane to their intracellular sites of metabolism in the mitochondria, where they enter the citric acid cycle. Because this

TABLE 1. Patient characteristics

	Infants (n = 51)	Children (n = 47)
Preoperative demographic characteristics	10 (5–14)*	49 (30–84)
Sex (M/F)	31/20	25/22
Body weight (kg)	6.2 (5.0–8.5)*	14.6 (10.9–20.3)
Qp/Qs	3.0 (2.2–3.7)*	1.8 (1.5–2.3)
RV/LV	0.58 (0.44–0.78)	0.47 (0.33–0.78)
LVEF (%)	70 (66–74)	69 (65–74)
Failure to thrive (%)	60.8*	21.3
Intraoperative clinical characteristics		
CPB time (min)	142 (127–151)	137 (108–154)
AoX time (min)	75 (63–83)	71 (56–85)
Lowest rectal temperature (°C)	28.0 (27.5–28.7)*	29.6 (28.4–30.3)

Data are presented as medians (interquartile range) or number of observations. Failure to thrive was defined as a patient body weight less than 2 SDs of the normal body weight. *Qp/Qs*, Pulmonary/systemic flow ratio; *RV/LV*, right ventricular/left ventricular systolic pressure ratio; *LVEF*, left ventricular ejection fraction; *CPB*, cardiopulmonary bypass; *AoX*, aortic crossclamp.

* $P < .01$ versus children.

protein features a low-molecular-weight and a cytosolic localization, it leaks out easily from the injured myocardium and immediately enters the blood circulation.^{5,6}

In this study we analyzed the severity of myocardial injury in pediatric cardiac surgery by measuring HFABP release and assessed the effects of age and ischemic time on the release of HFABP.

Methods

Patient Population

Only patients who had simple ventricular septal defects (VSDs) were included in this prospective study protocol to exclude bias in diagnosis and operative technique. From June 1999 through December 2001, 98 consecutive patients (51 infants <24 months of age and 47 children ≥24 months of age) underwent transatrial repair of VSD at Kobe Children's Hospital. This age criterion was selected from the median age of all patients. Preoperative characteristics are summarized in Table 1. No patients required preoperative respiratory or inotropic support. Approval from the local ethics committee was obtained, as was informed consent from all participating patients or their guardians.

Operative Procedure

Anesthesia was induced and maintained with fentanyl (50–100 µg/kg) and vecuronium bromide. Patients were ventilated with oxygen, air, and isoflurane, and ventilation was adjusted to maintain normocarbia. Cardiopulmonary bypass was instituted with an ascending aortic cannula, bicaval venous cannulas, and systemic ventricular venting. Systemic flow was maintained between 2.2 and 2.6 L · min⁻¹ · m⁻², and mean arterial pressure was maintained between 40 and 60 mm Hg. Mild or moderate systemic

TABLE 2. Composition of blood cardioplegia

Na ⁺ (mmol/L)	97.7 ± 1.2
K ⁺ (mmol/L)	18.7 ± 0.3
Cl ⁻ (mmol/L)	90.4 ± 0.8
Mg ²⁺ (mmol/L)	1.0 ± 0.01
Ca ²⁺ (mmol/L)	0.73 ± 0.01
pH	7.51 ± 0.01
HCO ₃ ⁻ (mmol/L)	17.4 ± 0.3
Hematocrit (%)	18.6 ± 0.6
Osmolarity (mOsm/L)	339 ± 3.1

All data are shown as means ± SEM for each composition.

hypothermia was used. After completion of VSD closure through the right atrium and an aortic declamping, rewarming was instituted. Cardiopulmonary bypass was discontinued with minimal inotropic support, and modified ultrafiltration was performed in a stable hemodynamic state.

Protocol of Myocardial Protection

Myocardial protection was achieved with intermittent cold blood cardioplegia with topical cardiac cooling in all patients. An initial dose of 300 mL/m² body surface area was initially infused into an aortic root at a pressure of 30 mm Hg to achieve cardiac arrest, with subsequent doses of 150 mL/m² body surface area infused every 20 minutes. In all patients, an additional 300 mL of warm (35°C) blood cardioplegic solution per square meter of body surface area was infused into the aortic root just before the aortic declamping.⁷ Blood cardioplegic solution⁴ was made by mixing hyperkalemic crystalloid solution⁸ with oxygenated blood in a 1:2 ratio and cooled to 9°C (Table 2). At the start of reperfusion, the aorta was partially declamped for 3 minutes, and the aortic root pressure was maintained at less than 30 mm Hg to reduce reperfusion injury. The aorta was then fully declamped. If ventricular fibrillation persisted beyond a few minutes after the aortic declamping, electrical defibrillation was applied to restore normal sinus rhythm.

Blood Samples and Biochemical Analysis

As markers of myocardial damage, serum concentrations of HFABP were measured at 6 points during the study protocol as follows: after induction of anesthesia and before skin incision and 0, 1, 2, 3, and 6 hours after the aortic declamping. Total release of HFABP was expressed as the area under the curve (AUC) of data monitored over time. All blood samples were collected through a radial arterial line and centrifuged immediately. The plasma was frozen and stored until biochemical analysis was performed. The serum HFABP level was measured by using a sandwich enzyme-linked immunosorbent assay with 2 distinct murine anti-HFABP monoclonal antibodies (Markit-M H-FABP; Dainippon Pharmaceutical Co Ltd, Osaka, Japan).⁹

Assessment of Clinical Outcome

The relationship between serum peak levels of HFABP and intraoperative and postoperative clinical variables was evaluated. Intraoperative variables included duration of cardiopulmonary bypass, aortic crossclamp (AoX) time, and lower rectal temperature. Postoperative variables included inotropic score, duration of intu-

TABLE 3. Clinical outcomes and their correlations with serum peak HFABP levels

	Infants			Children		
		<i>r</i> Value	<i>P</i> value		<i>r</i> Value	<i>P</i> value
Inotropic score*	460 (304–959)†	0.755	<.0001	192 (157–260)	0.585	<.0001
Intubation time (h)	24 (16–92)†	0.460	.0011	9 (6–16)	0.285	.0533
ICU stay (d)	6 (3–8)†	0.543	.0001	2 (2–4)	0.180	.2211

Data are presented as medians (interquartile range). *ICU*, Intensive care unit.

*Inotropic score is defined as the sum of doses of dopamine (in micrograms per kilogram per minute), dobutamine (in micrograms per kilogram per minute), and epinephrine (in micrograms per kilogram per minute $\times$ 100) multiplied by the number of hours that each drug was used. $P < .01$ versus children.

bation, and intensive care unit stay. Inotropic score was defined as follows: the sum of doses of dopamine (in micrograms per kilogram per minute), dobutamine (in micrograms per kilogram per minute), and epinephrine (in micrograms per kilogram per minute $\times$ 100) multiplied by the number of hours that each drug was used.¹⁰

Statistical Analysis

Database management and statistical analysis were performed with the Statview (version 5.0) software package (Abacus Concepts Inc). Normally distributed data are reported as means $\pm$ SEM; data that were not normally distributed are reported as medians with interquartile ranges. The methods of analysis included Mann-Whitney *U* tests for continuous variables and χ^2 tests for dichotomous parameters. The relationships between preoperative patient characteristics and preoperative serum HFABP levels and serum peak HFABP levels were analyzed with forward stepwise regression. Spearman rank correlation coefficients (2-tailed) were used to evaluate whether serum peak HFABP levels were correlated with operative clinical variables. HFABP-AUC and age showed some degree of positive skewness, and we therefore performed statistical analysis on the basis of logarithmic transformation of these variables to get an approximately normal distribution. Linear or non-linear regression analysis was performed to determine the relationship between HFABP-AUC and age. For this analysis, the age data were evaluated as continuous variables. In addition, to quantify the independent effects of age and ischemic time and to examine their interactive effect, we used a general linear regression model.¹¹

Results

Clinical Outcome

In the forward stepwise multiple regression analysis for all patients, there was no statistical correlation between preoperative patient characteristics and preoperative serum HFABP levels. Although age, body weight, preoperative pulmonary/systemic flow ratio (Qp/Qs), and failure to thrive in infants were significantly different from those in children (Table 1), preoperative Qp/Qs and failure to thrive did not correlate with serum peak HFABP levels after aortic declamping in the forward stepwise multiple regression. There was no statistical difference between infants and children regarding cardiopulmonary bypass and AoX time. No hospital deaths or major postoperative complications were recorded in this study. Transient electrocardiographic

abnormalities of minor ST-segment changes were observed in 20 patients but did not relate to the increase in serum levels of biochemical markers. The postoperative clinical data are shown in Table 3. Inotropic support was required with dopamine (range, 2–10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in all patients, dobutamine (range, 2–10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in 46 patients, or epinephrine (range, 0.03–0.1 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in 2 patients. All postoperative clinical variables were significantly better for children than for infants. In both infants and children, there was a good correlation between serum peak HFABP levels and inotropic score, with Spearman rank correlation coefficients of greater than 0.50 ($P < .01$ for each). Moreover, serum peak HFABP levels in infants correlated significantly with intubation time and intensive care unit stay.

Serial Changes in HFABP Levels

Serial changes in HFABP levels are shown in Figure 1. Before the operation, the baseline of serum HFABP levels in infants was not different from that in children. Serum HFABP levels at each point after aortic declamping in infants were significantly higher than those in children (median value, 234 vs 97 ng/mL at 0 hour, 455 vs 190 ng/mL at 1 hour, 186 vs 69 ng/mL at 2 hours, 107 vs 41 ng/mL at 3 hours, and 59 vs 22 ng/mL at 6 hours after aortic declamping, respectively; $P < .0001$). In both infants and children, serum HFABP levels reached the peak only 1 hour after aortic declamping.

Relationship Between Age and HFABP Release

In Figure 2, A, total HFABP release (*y*), as expressed by the AUC (HFABP-AUC) and age (*x*) in pediatric cardiac surgery are plotted on a logarithmic (ln) scale. The straight line is the least-square regression:

$$\ln(y) = a + b \ln(x) \quad (1)$$

where $a = 7.97 \pm 0.29$ and $b = -0.49 \pm 0.09$, with a correlation coefficient (*r*) of 0.738. A highly significant negative correlation was found between the logarithm of HFABP release and the logarithm of age ($P < .0001$). The

Figure 1. Serum levels of HFABP during the study protocol. Results are expressed as medians with interquartile ranges. *Preop*, Preoperative. * $P < .01$ versus serum HFABP levels at each point after aortic declamping in children.

dotted lines in Figure 2, A, show the confidence intervals and prediction limits for the regression lines.

Equation 1 can be rewritten as follows:

$$y = kx^b \quad (2)$$

where $k = \exp(a)$. Data were back-transformed to get the usual relationship in the original scale and plotted in Figure 2, B. The solid line in Figure 2, B, denotes equation 2, which is the nonlinear regression model of the data. It has been shown that the relationship between HFABP release and age follows a negatively accelerating power function.

Effect of Age and Ischemic Time on HFABP Release

The 2 subgroups were divided according to AoX time (group S, <75 minutes; group L, ≥75 minutes). The criterion of AoX time was selected from the median AoX time of all patients. Total HFABP release in infants was significantly greater than that in children (medians of 1133 vs 425 ng/mL, $P < .0001$). The HFABP-AUC level in group L was higher than that in group S (infants, medians of 1331 vs 873 ng/mL, $P = .005$; children, medians of 461 vs 368 ng/mL, $P = .011$). The HFABP-AUC level was dependent on AoX time. Moreover, the difference in HFABP release between the subgroups showed a trend toward a greater increase in infants than in children (Figure 3). The interaction between age and AoX time might cause this effect on HFABP release.

Interaction Between Age and Ischemic Time

Equation 3 represents a fitted general linear regression model for the logarithm of HFABP release (y), including the logarithm of age (x) as a main variable, AoX time (M) as a

modifying variable, and the interaction of both variables as follows:

$$\ln(y) = p + q\ln(x) + rM + s\ln(x)M \quad (3)$$

To estimate the effect of AoX time, M is a dummy variable defined as follows: $M = 0$ for group S, and $M = 1$ for group L. Table 4 shows the result of this analysis. In the model of equation 3, as well as equation 1, a highly significant negative correlation was found between the logarithm of age and the logarithm of HFABP release ($P < .0001$). Also, the logarithm of HFABP release correlated significantly with AoX time ($P = .0012$). There was a significant negative interaction with age and AoX time ($P = .0361$).

Equation 3 can be also rewritten as follows:

$$\ln(y) = (p + rM) + (q + sM)\ln(x)$$

$$y = Kx^L \quad (4)$$

where $K = \exp(p + rM)$ and $L = q + sM$. The amplitude and exponent parameter of this power function was dependent on AoX time. In Figure 4, HFABP-AUC (y) and age (x) are plotted by AoX time (M). It is clear that the difference in HFABP release between group S ($M = 0$) and group L ($M = 1$) showed a greater increase in infants than in children.

Discussion

Although serum HFABP concentration after cardiac surgery has only been reported in adult patients,^{12,13} we recently found that HFABP is also a rapid indicator for assessment of myocardial damage in pediatric cardiac surgery. Serum HFABP levels reached peak level at 1 hour after declamping, which was significantly earlier than serum creatine kinase isoenzyme MB or serum cardiac troponin T (TnT) levels. In addition, serum HFABP levels immediately after declamping correlated strongly with serum peak HFABP levels. The serum peak level of HFABP correlated with those of creatine kinase isoenzyme MB and TnT significantly. In multiple regression analysis, age, AoX time, the presence of a ventriculotomy, and the lowest hematocrit level during cardiopulmonary bypass were significant intra-variables that influenced the release of HFABP.⁴ In this series serum HFABP levels increased sharply from the baseline to the peak and reached the peak at 1 hour after aortic declamping. Ninety-eight percent of our patients reached their individual peak levels at this time point. These findings in infants and children agreed with those of the previous report.

In this study we showed that serum HFABP levels after pediatric cardiac surgery were significantly influenced by the age of the patients. It should be emphasized that infants (<24 months of age) displayed significantly greater amounts of serum HFABP than did children (≥24 months

Figure 2. Relationship between age and HFABP release. Results of linear regression analysis of the data on a logarithmic scale (A) and nonlinear regression analysis of the data in an original scale (B) are shown.

Figure 3. Effect of age and ischemic time on HFABP release.

of age) at each time point analyzed. Interestingly, we observed a negative power-law relationship between age and HFABP release. The validity of this relationship was demonstrated by a highly significant correlation between age and HFABP release in the ln-ln plot. Previously, Taggart and colleagues¹ have shown that the accumulation of TnT release shows a power-law relationship to age, as do our results relative to HFABP release. We can say with fair certainty that the younger the age of the patients, the more vulnerable are their myocardia to injury during definitive repair of congenital heart disease.

Generally, myocardial metabolic response to surgical stress seems greater in the younger heart. In addition to HFABP and TnT, Smolenski and coworkers¹⁴ reported a much greater release of lactate, phosphate, and purines in coronary sinus effluent in children aged 2 to 10 years

undergoing cardiac surgery than in adults. Alcaraz and associates¹⁵ showed that newborn patients displayed significantly greater amounts of serum interleukin 10 than did older children in response to cardiac surgery. These findings support the view that the immaturity or the fine sensibility of pediatric myocardium results in much more myocardial metabolic injury and a greater stress response. On the basis of this view, it is reasonable to postulate that surgical stress by ischemic time could promote a more biochemical response in infants than in children.

Ischemic time was also a highly significant explanatory variable for the release of HFABP. In both infants and children, there were positive correlations between ischemic time and HFABP release. We found that the ischemic time was the exponent and amplitude parameter of the power function between age and HFABP release. Interestingly, the effect of ischemic time on HFABP release was greater in infants than in children. This fact implied the presence of an interaction between age and ischemic time. Actually, our general linear model for HFABP release showed that myocardial damage was dependent on not only age and ischemic time but also on their interaction. We demonstrated statistically that the heart was more vulnerable to injury caused by myocardial ischemia in infants than in children.

Postoperative clinical outcomes were related to serum concentrations of HFABP after aortic declamping. In particular, we showed that inotropic support correlated significantly with serum peak HFABP levels in infants and children. In our study infants displayed significantly greater amounts of serum HFABP than did children after cardiac surgery, and the former had clinical outcomes that were not as good as those of children. It is also possible to say that

TABLE 4. Results of general linear model for the logarithm of HFABP release

Variable	Coefficient	SE	P value	95% CI
Ln (AGE)	−0.380	0.056	<.0001	−0.490 to −0.269
TIME	0.914	0.274	.0012	0.371 to 1.458
Ln (AGE) *TIME	−0.185	0.087	.0361	−0.357 to −0.012
Intercept	7.446	0.190	<.0001	7.070 to 7.823

HFABP, Heart fatty acid-binding protein; CI, confidence interval; Ln, logarithm; AGE, patient's age; TIME, aortic crossclamp time.

clinical outcomes in pediatric cardiac surgery are dependent on the age of the patient. Recently, definitive repair for patients with congenital heart disease tends to favor the younger patients because of the advances in surgical technique, myocardial preservation, and postoperative care. In our experience with VSD repair, the mean age of the patients was 4.4 ± 0.3 years between 1996 and 1998 versus 3.3 ± 0.2 years between 1999 and 2001. The percentage of patients aged less than 2 years of all patients with VSD repair was 30.4% between 1996 and 1998 versus 46.1% between 1999 and 2001. In this tendency it is important to manage perioperative care for pediatric patients with congenital heart disease, taking into consideration the dependence of the myocardial damage on age and ischemic time.

This study population was carefully selected to evaluate the effects of age and ischemic time on the release of HFABP in pediatric cardiac surgery. Because of this selectivity, the limitation of the study is an absence of patients with repair of congenital heart disease, except for a single VSD. Neonatal patients and patients with cyanosis were not included in the study. In addition, the analysis was limited with respect to the presence of large volume overload and pulmonary hypertension. The severity of VSD is certainly intensified by pulmonary hypertension, in which case patients often need surgical repair in infancy rather than in childhood. Although these factors did not correlate with the baseline of serum HFABP levels before the operation or serum peak HFABP levels after aortic declamping, further studies including the abovementioned patients are intended to better characterize HFABP in pediatric cardiac surgery. Another possible limitation is that postoperative clinical variables can be subjective according to the clinician managing the patient. Clinician bias is, however, unlikely to have been a confounding factor because the postoperative management was strictly in accordance with our unit protocol, and the results were not available to the clinician.

In conclusion, this study expands current knowledge on myocardial injury in pediatric cardiac surgery and demonstrates a pattern of HFABP release in infants and children with dependence on age and ischemic time. Our results indicated that the younger the age of patients, the more vulnerable are their myocardia to injury caused by ischemia during definitive repair of congenital heart disease. Therefore, we should manage perioperative care for pediatric

Figure 4. Relationship between age and HFABP release by ischemic time.

patients with congenital heart disease by taking into consideration the dependence of the myocardial damage on age and ischemic time.

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References

1. Taggart DP, Hadjinikolas L, Hooper J, Albert J, Kemp M, Hue D, et al. Effects of age and ischemic times on biochemical evidence of myocardial injury after pediatric cardiac operations. *J Thorac Cardiovasc Surg.* 1997;113:728-35.
2. Imura H, Caputo M, Parry A, Pawade A, Angelini GD, Suleiman MS. Age-dependent and hypoxia-related differences in myocardial protection during pediatric open heart surgery. *Circulation.* 2001;103:1551-6.
3. Carr LJ, VanderWerf QM, Anderson SE, Kost GJ. Age-related response of rabbit heart to normothermic ischemia: a 31P-MRS study. *Am J Physiol.* 1992;262:H391-8.
4. Hasegawa T, Yoshimura N, Oka S, Ootaki Y, Toyoda Y, Yamaguchi M. Evaluation of heart fatty acid-binding protein as a rapid indicator

- for assessment of myocardial damage in pediatric cardiac surgery. *J Thorac Cardiovasc Surg.* 2004;127:1697-702.
5. Schaap FG, van der Vusse GJ, Glatz JFC. Fatty acid-binding proteins in the heart. *Mol Cell Biochem.* 1998;180:43-51.
 6. Okamoto F, Sohmiya K, Ohkaru Y, Kawamura K, Asayama K, Kimura H, et al. Human heart-type cytoplasmic fatty acid-binding protein (H-FABP) for the diagnosis of acute myocardial infarction. Clinical evaluation of H-FABP in comparison with myoglobin and creatine kinase isoenzyme MB. *Clin Chem Lab Med.* 2000;38:231-8.
 7. Toyoda Y, Yamaguchi M, Yoshimura N, Oka S, Okita Y. Cardioprotective effects and the mechanisms of terminal warm blood cardioplegia in pediatric cardiac surgery. *J Thorac Cardiovasc Surg.* 2003;125:1242-51.
 8. Roe BB, Hutchinson JC, Ulliyot DJ, Smith DL. Myocardial protection with cold, ischemic, potassium-induced cardioplegia. *J Thorac Cardiovasc Surg.* 1977;73:366-70.
 9. Ohkaru Y, Asayama K, Ishii H, Nishimura S, Sunahara N, Tanaka T, et al. Development of sandwich enzyme-linked immunosorbent assay for the determination of human heart type fatty acid-binding protein in plasma and urine by using two different monoclonal antibodies specific for human heart type fatty acid-protein. *J Immunol Methods.* 1995;178:99-111.
 10. Kulik TJ, Moler FW, Palmisano JM, Custer JR, Mosca RS, Bove EL, et al. Outcome-associated factors in pediatric patients treated with extracorporeal membrane oxygenator after cardiac surgery. *Circulation.* 1996;94(suppl II):II63-8.
 11. Figueiras A, Domenech-Massons JM, Cadarso C. Regression models: calculating the confidence interval of effects in the presence of interactions. *Stat Med.* 1998;17:2099-105.
 12. Suzuki K, Sawa Y, Kadoba K, Takahashi T, Ichikawa H, Kagisaki K, et al. Early detection of cardiac damage with heart fatty acid-binding protein after cardiac operations. *Ann Thorac Surg.* 1998;65:54-8.
 13. Petzold T, Feindt P, Sunderdiek U, Boeken U, Fischer Y, Gams E. Heart-type fatty acid binding protein (hFABP) in the diagnosis of myocardial damage in coronary artery bypass grafting. *Eur J Cardiothorac Surg.* 2001;19:859-64.
 14. Smolenski RT, Swierczynski J, Narkiewicz M, Zydowo MM. Purines, lactate and phosphate release from child and adult heart during cardioplegic arrest. *Clin Chim Acta.* 1990;192:155-64.
 15. Alcaraz AJ, Sancho L, Manzano L, Esquivel F, Carrillo A, Prieto A, et al. Newborn patients exhibit an unusual pattern of interleukin 10 and interferon gamma serum levels in response to cardiac surgery. *J Thorac Cardiovasc Surg.* 2002;123:451-8.