ORIGINAL ARTICLE

Prognostic indicators after cardiac surgery in children and their relationship with the oxidative stress response

R. Gil-Gómez a, J. Blasco-Alonso b,d,*, R. Castillo-Martín c, G. Milano-Manso a,d

a Unidad de Gestión Clínica de Cuidados Críticos y Urgencias Pediátricos, Hospital Regional Universitario de Málaga, Málaga, Spain
b Sección de Gastroenterología y Nutrición Infantil, Unidad de Gestión Clínica de Pediatría, Hospital Regional Universitario de Málaga, Málaga, Spain
c Sección de Cirugía Cardiovascular Pediátrica, Hospital Regional Universitario de Málaga, Málaga, Spain
d Grupo Multidisciplinar de Investigación Pediátrica, Instituto de Investigación Biomédica de Málaga (IBIMA), Universidad de Málaga, Málaga, Spain

Received 26 September 2014; accepted 23 January 2015
Available online 27 July 2015

KEYWORDS
Cardiac surgical procedures;
Extracorporeal circulation;
Cardiopulmonary bypass;
Interleukins;
Systemic inflammatory response syndrome;
Paediatrics

Abstract

Objectives: To analyse the trend in lipid peroxidation and antioxidant response as key markers of oxidative stress after paediatric cardiovascular surgery, and compare them with other internationally accepted clinical prognostic indicators.

Patients and methods: A prospective study was conducted on 30 children aged one month to 14 years, weight > 5 kg, undergoing cardiopulmonary bypass surgery. Blood samples were taken just before the intervention, immediately after surgery, and after 18-20 h. Cell membrane lipid peroxidation was analysed by quantifying malondialdehyde, as well as measuring total glutathione (oxidised and reduced), as representatives of antioxidant response. An analysis was also performed on clinical variables for establishing a score for the systemic inflammatory response syndrome associated with cardiopulmonary bypass.

Results: The study included 30 children with a mean age of 4.1 years old (interquartile range [IQR]: 2.7; 8.0). Of these, 62.1% were girls. The standard deviation of the median weight was −0.39 (IQR: −0.76; 0.24), the median height was −0.22 (IQR: −0.74; 0.27), and the median BMI was −0.43 (IQR: −1; 0.45). The final surgery times were divided into 2 parts: total time of extracorporeal circulation, with a mean of 79 min (IQR: 52.5; 125.5), and the clamping time, a measurement included in the previous figure with a mean value of 38.5 min (IQR: 22; 59). Malondialdehyde increased and glutathione decreased in postoperative time, with clear, statistically significant direct correlation between time of extracorporeal circulation and percentage decrease in total glutathione between preoperative and immediate postoperative time, and a

* Corresponding author.
E-mail address: javierblascoalonso@yahoo.es (J. Blasco-Alonso).

2341-1929/© 2014 Sociedad Española de Anestesiología, Reanimación y Terapéutica del Dolor. Published by Elsevier España, S.L.U. All rights reserved.
decline between the preoperative and late postoperative. There was a statistical correlation between total glutathione levels at 18–20 h postoperatively and the duration of mechanical ventilation and inflammatory systemic response syndrome.

Conclusions: Surgery with extracorporeal circulation performed in children activates inflammatory mediators, being maximum after aortic clamping, and improving after the first 24 h. The level of oxidative stress activation depends on surgical times. The development of systemic inflammatory response syndrome is associated with longer duration of mechanical ventilation, longer stay in intensive care, higher scores in the Aristotle model and longer surgical times. Those who do not meet criteria for inflammatory response have higher levels of glutathione in first 24 h.

© 2014 Sociedad Española de Anestesiología, Reanimación y Terapéutica del Dolor. Published by Elsevier España, S.L.U. All rights reserved.

PALABRAS CLAVE
Cirugía cardiaca; Circulación extracorpórea; Baipás cardiopulmonar; Interleucinas; Síndrome de respuesta inflamatoria sistémica; Pediatría

Indicadores pronósticos clínicos en el posoperatorio de cirugía cardiovascular pediátrica y su relación con la cinética del estrés oxidativo

Resumen
Objetivos: Analizar la evolución de parámetros de estrés oxidativo en el posoperatorio de cirugía cardiovascular pediátrica y correlacionarlos con diferentes indicadores clínicos pronósticos.

Material y métodos: Treinta niños, de entre un mes y 14 años, sometidos a circulación extracorpórea. Se obtuvieron muestras preoperatoria, posoperatoria inmediata y tras 18–20 h. Se analizó la capacidad de peroxidación lipídica de las membranas celulares mediante la cuantificación de productos de reacción con el ácido tiobarbituríco, cuyo principal representante es el malondialdehído; se cuantificó el contenido celular de glutatión total, oxidado y reducido (representantes de la respuesta antioxidante). Se analizaron las variables clínicas que permitieran establecer una puntuación para el síndrome de respuesta inflamatoria sistémica asociado a circulación extracorpórea.

Resultados: Treinta pacientes con una mediana de edad de 4,1 años (rango intercuartílico [RIC]: 2,7; 8,0); el 62,1% eran niñas; mediana de desviaciones estándar de peso —0,39 (RIC: —0,76; 0,24), de talla —0,22 (RIC: —0,74; 0,27) y de IMC —0,45 (RIC: —1; 0,45). Mediana de tiempo quirúrgico 79 min (RIC: 52,5; 125,5), mediana de pinzamiento 38,5 min (RIC: 22; 59). Aumentó el malondialdehído y disminuyó el glutatión en ambos momentos posoperatorios, con clara correlación directa, estadísticamente significativa, del tiempo de circulación extracorpórea con el porcentaje de descenso de glutatión total entre preoperatorio y posoperatorio inmediato y entre el preoperatorio y el posoperatorio tardío. Hubo una correlación estadística entre los niveles de glutatión total tras 18–20 h posoperatorias y el tiempo de duración de la ventilación mecánica y la pertenencia al grupo de síndrome de respuesta inflamatoria sistémica.

Conclusiones: La circulación extracorpórea activa mediadores inflamatorios, máximo tras el pinzamiento aórtico, mejorando tras 24 h, siendo dependiente de los tiempos quirúrgicos. El desarrollo de respuesta inflamatoria está asociado a una mayor duración de la ventilación mecánica, una estancia más prolongada en Cuidados Intensivos, puntuaciones mayores del Modelo de Aristóteles y tiempos más largos quirúrgicos. Los que no cumplen criterios de respuesta inflamatoria tienen más niveles de glutatión en el posoperatorio tardío.

© 2014 Sociedad Española de Anestesiología, Reanimación y Terapéutica del Dolor. Publicado por Elsevier España, S.L.U. Todos los derechos reservados.

Introduction

Over the last 20 years, extensive evidence has come to light to support the hypothesis that production of oxidants and reactive oxygen species (ROS) in the post-ischaemic heart is considerably elevated by different types of interaction between cardiac and endothelial cells. 1 Although different pathophysiological mechanisms could explain the reperfusion injury, the most widely accepted theory links oxidative stress with production of reactive oxygen species. 2,3

ROS-induced lipid peroxidation is a well-known cause of cell injury in which the lipid peroxidation of polyunsaturated fatty acids produces reactive aldehydes such as
malondialdehyde (MDA).\textsuperscript{4} Determination of these reactive aldehydes in blood or tissue is a good marker of lipid peroxidation, and therefore an indirect indication of ROS levels. Glutathione peroxidase inhibits the formation of new ROS by neutralising the peroxides that react with transition metals to give rise to ROS. This enzyme is a catalyst for glutathione oxidation by fatty acid hydroperoxide. Glutathione reductase complements this process by catalysing the reduction of oxidised glutathione and restoring the glutathione peroxidase substrate.\textsuperscript{7}

Heart surgery, and specifically cardiopulmonary bypass (CPB), is an ideal context for studying ischaemia-reperfusion injury, as these processes can be reproduced, with long-lasting ischaemia and controlled reperfusion.\textsuperscript{5} We hypothesised that the kinetics of oxidative stress and lipid peroxidation in children undergoing heart surgery correlates with clinical and analytical variables, and that these can predict postoperative evolution and help improve management of ischaemic reperfusion injury in subsequent interventions.

The aim of this study was to analyse the evolution of lipid peroxidation and antioxidant response parameters as the main markers of post-heart surgery oxidative stress in children, and to correlate these with various internationally accepted clinical prognosis indicators.

Material and methods

Inclusion criteria were: patients younger than 15 years, weighing over 5 kg, who underwent congenital heart surgery with CPB and were admitted to our Paediatric Intensive Care Unit (ICU) in a tertiary level hospital in the South of Spain. Patients with any clinical situation that could mask oxidative or inflammatory stress, patients with an autoimmune disease, patients undergoing emergency surgery, and patients with sepsis were excluded from the study. Patients weighing > 5 kg but with preoperative baseline haemoglobin levels under 10 g/dL were also excluded.

The lipid peroxidation capacity of cell membranes was determined by quantification of thiobarbituric acid reactive substances, mainly MDA, in nmoles/mg of protein. Total glutathione levels were determined by spectrophotometry, and the results expressed in nmoles/mg of protein.

Samples for analysis were taken at 3 times: time 1, or preoperative (PRE): immediately prior to induction, and prior to thoracotomy; time 2, or immediate postoperative (PO1): at least 1 h following aortic clamping; and time 3, or late postoperative (PO2): 18–20 h post-surgery.

The scientific community now has access to an international risk stratification model for cardiovascular surgery, the Aristotle Model, published by Lacour-Gayet et al. in 2004.\textsuperscript{7} In this model, the authors introduce the concept of complexity, which is the sum of the potential for 30-day mortality together with potential morbidity (length of stay in the ICU) and the anticipated technical difficulty of the procedure. Complexity is a constant value at a given time for a procedure in a given patient, regardless of the type and geographical location of the hospital where they receive surgery. Complexity is evaluated in 2 scoring steps: basic score and comprehensive score. Each factor is scored according to its contribution towards mortality, morbidity and technical difficulty. The basic score is only adjusted for the complexity of procedures. The sum of the mean mortality, morbidity and technical difficulty scores gave the final basic score for each procedure. The scale ranges from 1.5 to 15 points, and 4 risk categories were defined. The comprehensive step introduces the notion of complexity adjusted to patient characteristics. Two complexity factors were included: procedure-dependent factors (anatomical factors, associated procedures, age [6 groups]), and procedure-independent factors (general factors, clinical factors, extra-cardiac factors, surgical factors). All factors met the following requirements: precisely quantifiable, easily available, agreed by majority, and verifiable.\textsuperscript{8} Based on the foregoing, the comprehensive score adds two levels of complexity to a maximum basic score of 15 (1: 1.5–5.9 points; 2: 6.0–7.9 points; 3: 8.0–9.9 points; 4: 10.0–15.0 points), level 5 (15.1 to 20 points) and level 6 (20 to 25 points).

The hourly dose of dopamine, dobutamine, adrenaline, norepinephrine, milrinone and vasopressin was recorded over the first 48 h. We also calculated the inotropic score (IS) described by Wernovsky et al.,\textsuperscript{9} and extended this formula, as described by Gales et al.,\textsuperscript{10} to include other vasoactive agents commonly used in modern clinical practice. This extended formula was used to define a vasoactive and inotropic score (VIS). We elected to use coefficients of milrinone, norepinephrine and vasopressin, which convert these elements into whole numbers, and to give each drug the same weight in the formula.

\[
\text{Wernovsky IS} = \frac{\text{dopamine(mcg/kg/min)}}{10} + \frac{\text{dobutamine(mcg/kg/min)}}{10} + \frac{\text{adrenaline(mcg/kg/min)}}{100}
\]

\[
\text{VIS} = \text{IS} + 10 \times \frac{\text{milrinone(mcg/kg/min)}}{10000} + 100 \times \frac{\text{vasopressin(U/kg/min)}}{100} + 100 \times \frac{\text{noradrenaline(mcg/kg/min)}}{1000}
\]

We analysed clinical variables that would allow us to establish a score for cardiopulmonary bypass-induced systemic inflammatory response syndrome (CPB-SIRS)\textsuperscript{11} in the first 3 days post surgery, which is the period of most intense inflammatory activity. For this purpose, we gave 1 point to each of the following altered criteria: fever ($\geq 38^\circ$C), haemodynamic dysfunction (IS $> 20$), pulmonary dysfunction ($\text{PaO}_2/\text{FiO}_2$ ratio $< 300$), kidney dysfunction (creatinine increase $> 20$%) and clinical (chest tube drainage above the 3rd quartile with respect to the total sample) and radiological endothelial dysfunction (pre- and postoperative interstitial thickening $> 50$% according to the radiological index). This gave a score ranging from 0 to 6, and enabled us to divide the sample into 2 groups, based on the number of variables with altered parameters.\textsuperscript{12} Group 1 included all patients with a score of 3 or more, considered to be presence of CPB-SIRS. Group 2 consisted of patients with a top score of 3, i.e., no CPB-SIRS.

Neurological dysfunction was defined as the presence of cerebral vascular injury (on imaging), seizures, or cerebral oxygen saturation <40%.
To correlate oxidative stress with clinical outcome variables, we evaluated a new set of variables representing the percentage changes in ROS variables between PRE and PO1 (called \( \text{p1} \)), between PO1 and PO2 (called \( \text{p2} \)), and between PRE and PO2 (called \( \text{p3} \)).

The morbidity marker was defined as a stay of at least 1 week in the UCI. Surgical technical difficulty was defined as a CPB time of 120 min. The comprehensive Aristotle scores were correlated with mortality, morbidity and technical difficulty.

We performed a descriptive analysis with point estimation and a 95% confidence interval. We treated continuous variables as mean plus standard deviation or median and interquartile range (IQR), according to their distribution. Categorical variables were expressed as frequency and percentage. We performed a multivariate analysis with linear regression to identify predictor variables, adjusted for possible confounding factors and interactions. Risk factors for CPB-SIRS were compared using the Chi-square \((\chi^2)\) and Student’s \(t\) tests. In the case of categorical variables, we calculated the relative risks with their respective 95% confidence intervals. We also performed multiple regression, taking CPB-SIRS as the dependent variable. We used Pearson’s \(\chi^2\) test and the Mann–Whitney test to compare patients with or without CPB-SIRS in terms of outcomes.

We performed a descriptive statistical analysis by calculating the mean, standard deviation and coefficient of variation (CV) for the sample as a whole, and for each individual patient. The intra-individual CV was calculated as the mean of each subject’s CV, while the inter-individual CV was calculated on the basis of the CV of all the individual values of the whole sample. Pearson’s correlation coefficient was calculated to measure the strength of association between variables, and the corresponding hypothesis was tested to ascertain significance.

We used ROC curves, a global measure across all cut-off values, to select a clinical prognostic indicator for the presence of CPB-SIRS. The selection was based on analysing the area under the curve, ranging from 0.5 (test with no diagnostic discriminatory ability) and 1 (the ideal diagnostic value). To interpret the ROC curves, we defined an area under the curve of >0.75 as a good test, and an area of >0.9 as a very good or excellent test.\(^\text{14}\)

The project was approved by the Independent Ethics Committee of the Hospital Regional Universitario of Malaga. We adhered to all the principles of the Declaration of Helsinki (updated in 2008), the statement of ethical principles for medical research, in respect of the human subjects and biological material involved in the study.

**Results**

The study sample comprised 30 children (Fig. 1), recruited consecutively over a period of 10 months (some subjects were excluded on the basis of the study criteria and due to problems in extracting or processing samples). The median age of the patients was 4.1 years (IQR: 2.7–8.0); 19 were girls (63.3%). The surgery types were as follows: 11 closures of atrial septal defects, 2 pulmonary stenosis interventions, 3 total corrections of tetralogy of Fallot, 3 corrections of atroventricular canal defect, 6 closures of ventricular septal defects, 2 aortic stenosis interventions, 2 corrections of univentricular hearts (one using the Glenn technique and the other using the Fontan technique), and 1 surgical correction of transposition of the great arteries.

Duration of surgery was divided into two variables: total duration of CPB, with a median of 79 min (IQR: 52.5–125.5), and aortic clamping time, which is included in the former, with a median of 38.5 min (IQR: 22–59).

Table 1 shows that MDA production was significantly increased and different states of glutathione significantly reduced at each postoperative time point (PO1 and PO2) compared with baseline (PRE). The Friedman nonparametric test was used to compare the values of the antioxidant system (total glutathione, oxidised and reduced) and lipid peroxidation (MDA), taken at 3 different time points. The differences were statistically significant \((p < 0.001)\). After multiple comparisons using the Wilcoxon test and application of the Bonferroni correction, MDA and GST were found to differ significantly \((p < 0.016)\) at the 3 time points.

Table 2 shows the main clinical variables. Median UCI stay was 2.0 days (IQR: 2.0; 7.25), with a median duration of

---

**Table 1** Antioxidant defence (total glutathione) and lipid peroxidation (malondialdehyde) variables.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>GST (nmol mg of protein)</th>
<th>MDA (nmol mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.56 ± 0.77</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>2.56 ± 0.71(^a)</td>
<td>0.21 ± 0.09(^a)</td>
</tr>
<tr>
<td>3</td>
<td>3.25 ± 0.66(^b,c)</td>
<td>0.18 ± 0.06(^b,c)</td>
</tr>
</tbody>
</table>

\(^a\) Difference between time 1 and 2.

\(^b\) Difference between time 1 and 3.

\(^c\) Difference between time 2 and 3.
mechanical ventilation of 6.0 h (IQR: 4.75; 10.5). In terms of haemodynamics, 6 patients received 2 or more vasoactive drugs; only 2 presented VIS > 20. Three patients presented neurological dysfunction, and 2 showed pulmonary dysfunction. Fourteen patients presented kidney dysfunction; renal replacement therapy was needed in only 2 of these. Fever >38 °C was detected in 2 cases, and 3 children were diagnosed with sepsis in the first 5 days post-surgery. Significant arrhythmia (AV block and nodal rhythms) was seen in 5 patients. Three children met the criteria for CPB-SIRS (group i). Mean basic and comprehensive Aristotle score was in the level 2 range. In the first 8 h, the mean drainage amount was 1.0 ml/kg/h (IQR: 0.38; 1.74), which fell to 0.4 ml/kg/h over the following 10 h (IQR: 0.18; 0.95). This finding was statistically significant (p = 0.001), and shows a good correlation between these 2 periods (Pearson’s r 0.55, p = 0.002). In this 18-h period, only 8 patients were above the third quartile with respect to the total sample (clinical endothelial dysfunction).

A total of 23.3% of patients met the criteria for the CPB time >120 min technical difficulty indicator. This showed a statistically significant correlation with the comprehensive Aristotle score (Spearman’s rho 0.66, p < 0.001). Nine out of the 30 study patients stayed more than 6 days in the UCI after surgery. This technical difficulty indicator differed greatly between patients classified as CPB-SIRS and non-CPB-SIRS patients (Table 3), and also showed a statistically significant correlation with the comprehensive Aristotle score (Spearman’s rho 0.63, p < 0.001) and with CPB time (rho 0.50, p = 0.006).

A statistically significant correlation was also found between the comprehensive Aristotle score and the CPB-SIRS score, albeit slightly lower in this case (rho 0.48, p = 0.009). No differences in mean age were found between patients presenting CPB-SIRS criteria and non-CPB-SIRS patients. However, in these 2 patient groups differences in CPB time, length of Paediatric ICU stay, time (hours) on mechanical ventilation, and both basic and comprehensive types of Aristotle score were statistically significant (Table 3).

Oxidative stress markers were studied in relation to the different clinical variables defined in the study. Statistically significant correlations were found between total glutathione levels at 18-20h postoperative, time on mechanical ventilation, and CPB-SIRS patient. Likewise, in terms of variables showing the differential increase between different analysis times, significant correlation was found between percentage reduction in total glutathione during PRE and PO1 (surgery times), and between reduction during PRE and PO2 (CPB-SIRS, surgery times, and length of stay). Fig. 2 shows a box chart comparing GST3 and GST_p3 between CPB-SIRS and non-CPB-SIRS patients.

Linear regression was used to evaluate independent predictors for the different clinical indicators studied. Those with a true impact as individual factors are shown in Table 4, and were principally those with a B coefficient greater that «+2» o «−2».

Intra-individual coefficients of variation were lower than inter-individual values for all study parameters.

Using ROC curves to select a clinical prognostic indicator for CPB-SIRS yielded good scores for IS, VIS, basic Aristotle, and comprehensive Aristotle (Fig. 3), all of which had a sensitivity of 100% for the corresponding cut-off values. IS, meanwhile, had a specificity of 96.3%, with values above 7; VIS had a specificity of 92.6%, with a cut-off value of 16.5. In terms of basic Aristotle, a score of 7.25 had a specificity of 80% and a comprehensive Aristotle score of 9 had a

### Table 2  Values for the main clinical variables studied.

<table>
<thead>
<tr>
<th></th>
<th>Drainage first 8 h (ml/kg/h)</th>
<th>Drainage second 10 h (ml/kg/h)</th>
<th>Stay (days)</th>
<th>Time on MV (h)</th>
<th>Inotrope score</th>
<th>Vasoactive inotrope scoring</th>
<th>Basic Aristotle</th>
<th>Comprehensive Aristotle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.11</td>
<td>0.61</td>
<td>5.5</td>
<td>24.6</td>
<td>2.13</td>
<td>7.20</td>
<td>6.14</td>
<td>7.07</td>
</tr>
<tr>
<td>Median</td>
<td>1.00</td>
<td>0.40</td>
<td>2.0</td>
<td>6.0</td>
<td>0.00</td>
<td>0.00</td>
<td>6.00</td>
<td>6.15</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.84</td>
<td>0.49</td>
<td>7.6</td>
<td>63.1</td>
<td>6.10</td>
<td>10.59</td>
<td>2.69</td>
<td>3.85</td>
</tr>
<tr>
<td>25th percentile</td>
<td>0.38</td>
<td>0.18</td>
<td>2.0</td>
<td>4.7</td>
<td>0.00</td>
<td>0.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>75th percentile</td>
<td>1.74</td>
<td>0.95</td>
<td>7.2</td>
<td>10.5</td>
<td>0.00</td>
<td>12.00</td>
<td>7.88</td>
<td>8.00</td>
</tr>
</tbody>
</table>

### Table 3  Factors correlated with development of cardiopulmonary bypass-associated systemic inflammatory response syndrome. Comparison of patients meeting CPB-SIRS criteria and non-CPB-SIRS patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CPB-SIRS</th>
<th>No CPB-SIRS</th>
<th>p</th>
<th>Spearman’s correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rho</td>
<td>Rho</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>4.1 ± 4.6</td>
<td>5.3 ± 3.4</td>
<td>ns</td>
<td>0.493</td>
</tr>
<tr>
<td>Duration of CPB (min)</td>
<td>205.3 ± 81.8</td>
<td>79.6 ± 38.7</td>
<td>0.01</td>
<td>0.516</td>
</tr>
<tr>
<td>Clamp time (min)</td>
<td>89.0 ± 90.7</td>
<td>39.0 ± 22.5</td>
<td>ns</td>
<td>0.511</td>
</tr>
<tr>
<td>ICU stay (days)</td>
<td>21.6 ± 17.7</td>
<td>3.7 ± 2.8</td>
<td>0.005</td>
<td>0.433</td>
</tr>
<tr>
<td>Time on MV (h)</td>
<td>152.0 ± 163.3</td>
<td>10.5 ± 17.9</td>
<td>0.006</td>
<td>0.495</td>
</tr>
<tr>
<td>Basic Aristotle</td>
<td>10.3 ± 3.6</td>
<td>5.6 ± 1.2</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>Comprehensive Aristotle</td>
<td>14.3 ± 3.8</td>
<td>6.1 ± 2.8</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>
higher specificity of 88%; CPB time in excess of 135 min was a predictor of CPB-SIRS, with a specificity of 88%.

**Discussion**

The relationship between oxidative stress and heart surgery is still at the research stage. Most studies to date have been performed in animal models. The physiological levels of oxidation products or ROS in children are unknown. Neither do we know with any certainty which enzymatic redox systems have the greatest influence on systemic inflammatory response, nor how they function physiologically in human beings.

Heart surgery is an ideal context for studying ischaemia–reperfusion injury, as these processes can be reproduced, with long-lasting ischaemia and controlled reperfusion following aortic clamping. CPB is not a pure model, as it is "masked" by the effects of anaesthesia, hypothermia, cardioplegia, and the CPB process itself, which can be confounding factors. In our model, this masking factor is overcome by making the first post-induction determination before opening the sternum.

Our study was performed in paediatric patients, a group characterised by a significant difference in the degree of maturity of enzymatic processes. This is a major drawback, and can make comparisons with studies performed in other age groups impossible. However, this problem has been overcome to a certain extent in our study by limiting the patient age group. Little is currently known about the role of oxidative stress in healthy children.

In our patients, reperfusion was accompanied by the formation of ROS, a circumstance that was confirmed by directly measuring oxygen radical levels or the release of cardiac glutathione. However, exposure to oxidants does not necessarily imply damage from these agents. In fact, the glutathione pathway is one of the main mechanisms of the endogenous antioxidant defence system. ROS, by oxidising extracellular glutathione oxidation, are effectively
inactivated. As the oxidation attack continues or intensifies, lipid membrane peroxidation occurs, which in turn induces organelle dysfunction and can culminate in ultrastructural damage. In the paediatric CPB model used in this study, we observed oxidative stress and lipid peroxidation, both of which reached maximum levels after aortic clamping and improved after 24 h (increased oxidative and reduced antioxidant activity) without returning to normal values at time 3, after 18–20 h. We also observed significant differences between PRE and PO2 values, which even led to a significant correlation with prognostic indicators, such as the presence of CPB-SIRS. Oxygen radicals are highly toxic metabolites that lead to lipid peroxidation and cell damage. This in turn leads to capillary leakage and the production of shock-induced adhesion molecules. This process can activate macrophages in the liver and cause the synthesis, when in a state of shock, of proinflammatory mediators such as tumour necrosis factor and interleukin. Lipid peroxidation is an important cause of oxidative damage of cell membranes and eventually cell death. MDA, an end-product of lipid peroxidation, is a good indicator of oxidative damage. Glutathione is crucial as a cell defence mechanism against oxidative stress.

In addition to its antioxidant activity, GST plays an important role in transmembrane amino acid transport, protein synthesis and degradation, gene regulation, and cell redox regulation. GST mobilisation has been shown to increase in septic animals, with increased usage, a process that was also observed in our model of CPB. Therefore, the possibility of modulating the availability of GST may be an attractive strategy, but studies with clinically relevant outcomes have yet to be conducted.

The technical difficulty indicator (CPB time > 120 min), being a postoperative datum, correlated significantly with the comprehensive Aristotle score, which is a measure of complexity calculated prior to surgery.

In our series, the median ICU stay was short (<7 days), with early extubation. The longer mean stay correlated very well with both the CPB time and with the comprehensive Aristotle score, and above all with the presence of CPB-SIRS.

The technical advances of modern medical practice have led to increasingly complex and costly treatment strategies, such as CPB. Until a few years ago, surprisingly little information was available for objectively estimating the quality of the medical care given, despite the number of resources involved. Using data available at that time, it was impossible to objectively compare the efficacy, efficiency and quality of medical and hospital services. This is why systems for stratifying patients have been developed in recent years in order to explain and understand treatment outcomes. The Aristotle score system is one of the efforts made in this direction.

Difficulties have been encountered in analysing the frequency of kidney function problems following surgery, due to the use of different diagnostic criteria used in different groups. In adults, Tuttle et al. studied a population undergoing CPB using a kidney failure criterion similar to that used in our study (25% increase in creatinine), and reported an incidence of 42%. In our study, the incidence was 46.6%, and was an important criterion in the CPB-SIRS score, as 21.4% of patients with kidney failure presented CPB-SIRS, while none with normal kidney function were diagnosed with CPB-SIRS.

We found a 10% incidence of CPB-SIRS in a paediatric population scheduled for elective surgery in which a large number of other preoperative risk factors had been excluded. Other studies in paediatric populations have evaluated SIRS. In these, the diagnostic criterion used was adapted from its definition in clinical patients, and the authors reported good specificity, although sensitivity was still inadequate (effect of surgical factors and treatment given in the ICU). In the CPB-SIRS group, outcome was worse relative to length of stay in the ICU. In patients presenting CPB-SIRS, mechanical ventilation time was longer, Aristotle score was higher, and CPB time was also longer. Predictive factors for CPB-SIRS, meanwhile, are longer CPB.

### Table 4: Independent predictors for the different clinical parameters. Multiple linear regression.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Predictor variables</th>
<th>p</th>
<th>B</th>
<th>95% Confidence interval for B</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU stay</td>
<td>Time on MV</td>
<td>0.014</td>
<td>−0.10</td>
<td>−0.18,−0.02</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>0.036</td>
<td>−0.79</td>
<td>−1.53,−0.06</td>
</tr>
<tr>
<td></td>
<td>CPB-SIRS</td>
<td>&lt;0.001</td>
<td>40.28</td>
<td>23.21,57.35</td>
</tr>
<tr>
<td>Neurological dysfunction</td>
<td>Sepsis</td>
<td>0.002</td>
<td>−2.75</td>
<td>−4.26,−1.23</td>
</tr>
<tr>
<td></td>
<td>ICU stay</td>
<td>0.007</td>
<td>0.07</td>
<td>0.02,0.12</td>
</tr>
<tr>
<td></td>
<td>Time on MV</td>
<td>0.002</td>
<td>0.01</td>
<td>0.006,0.02</td>
</tr>
<tr>
<td></td>
<td>VIS</td>
<td>0.002</td>
<td>−0.04</td>
<td>−0.06,−0.01</td>
</tr>
<tr>
<td></td>
<td>CPB-SIRS</td>
<td>0.009</td>
<td>−1.58</td>
<td>−2.68,−0.49</td>
</tr>
<tr>
<td>VIS</td>
<td>Sepsis</td>
<td>&lt;0.001</td>
<td>−62.08</td>
<td>−75.74,−48.42</td>
</tr>
<tr>
<td></td>
<td>ICU stay</td>
<td>0.001</td>
<td>1.53</td>
<td>0.76,2.30</td>
</tr>
<tr>
<td></td>
<td>Time on MV</td>
<td>&lt;0.001</td>
<td>0.30</td>
<td>0.22,0.39</td>
</tr>
<tr>
<td>Neurological dysfunction</td>
<td>CPB-SIRS</td>
<td>0.001</td>
<td>−14.36</td>
<td>−21.69,−7.03</td>
</tr>
<tr>
<td>Pulmonary dysfunction</td>
<td>&lt;0.001</td>
<td>17.54</td>
<td>11.46</td>
<td>23.63</td>
</tr>
<tr>
<td></td>
<td>ICU stay</td>
<td>&lt;0.001</td>
<td>0.03</td>
<td>0.02,0.05</td>
</tr>
<tr>
<td></td>
<td>Time on MV</td>
<td>&lt;0.001</td>
<td>0.007</td>
<td>0.005,0.009</td>
</tr>
<tr>
<td>Neurological dysfunction</td>
<td>CPB-SIRS</td>
<td>0.007</td>
<td>−0.29</td>
<td>−0.49,−0.09</td>
</tr>
</tbody>
</table>

Data expressed as B coefficient (95% CI) and level of significance p. Significance level is p < 0.05.
longer ICU stay, longer (hours) mechanical ventilation time, and higher comprehensive Aristotle score.

Better antioxidant response at 24 h post-surgery (measured by total glutathione levels) is predictive of less likelihood of developing CPB-SIRS and shorter mechanical ventilation time.

Because of the high prevalence of milrinone administration, in our series VIS score was more closely correlated with other clinical variables and predictors. Significant arrhythmia was defined as arrhythmia secondary to surgical complications or low cardiac output. This was observed in only a few of our patients. The drainage rate was reasonable well controlled in the first 24 h. However, median and quartile values enabled us to determine the upper quartile group of children, which were also those presenting clinical endothelial dysfunction.

Multiple linear regression analysis enabled us to evaluate the real factors affecting clinical indicators. These were found to be length of ICU stay, mainly for CPB-SIRS, neurological dysfunction due to sepsis and CPB-SIRS, and VIS score due to sepsis, neurological or pulmonary dysfunction.
The use of ROC curves established cut-off values for independent variables that can be used to predict onset of CPB-SIRS, with sensitivity and specificity adjusted for the model.

Given that oxidative stress abnormalities are also found in heart surgery, it would be interesting to determine their clinical repercussions, if any, and to find technological, pharmacological or surgical methods for minimising these alternations. Investigation of oxidative stress in heart surgery offers a unique opportunity to make observations in clinically relevant conditions, given that knowledge to date has been derived from animal models. Such information would have important therapeutic implications, because preventing this phenomenon would afford greater myocardial protection and improve postoperative outcomes.

Ischaemia and reperfusion contribute to the oxidative stress and SIRS occurring during CPB. Proinflammatory cytokines can explain many of the signs of shock, such as respiratory failure, capillary leakage, depressed myocardial function, kidney failure and cellular ischaemia. Anti-inflammatory mediators, such as IL-10, are thought to play an important part in the down-regulation of the early inflammatory response. Some studies indicate the benefits of early systemic release of IL-10 following injury and shock.21

Conclusions

Onset of CPB-SIRS is associated with longer time under mechanical ventilation, longer stay in the ICU, higher Aristotle score, and longer CPB time. Longer CPB time, in turn, correlates with greater glutathione deficiency at 18–20 post-surgery. This is also seen in the percentage increase in glutathione between PRE and PO2. Patients not meeting CPB-SIRS criteria have higher glutathione level at time 3.

Despite the limitations inherent to such a small sample size, the findings of this study are important and provide a convincing argument in favour of a far-reaching intervention protocol due to the potential preventive and therapeutic strategies. These include the possibility of increasing antioxidant enzyme activity by pharmacological means both before starting CPB and during the first 12–18 after disconnection.

It is important to note that molecular biology is revolutionising medicine and our ability to evaluate to impact of variability on the characterisation of diseases and perioperative outcomes.

Funding

This study was funded in part by a research grant from the SEGHN, under the 3rd Nestlé Paediatric Nutrition Research Grant programme in 2009.

Conflict of interest

The authors declare they have no conflicts of interest.

References