Circulating histones for predicting prognosis after cardiac surgery: a prospective study

Hongxiang Gao, Naipu Zhang, Fangfang Lu, Xindi Yu, Limin Zhu, Xi Mo, and Wei Wang

OBJECTIVES: The objective of this study was to assess the perioperative changes in circulating histones and their relationships with other biomarkers and clinical outcomes after cardiac surgery with cardiopulmonary bypass (CPB) in patients.

METHODS: Forty-eight patients with congenital cardiac diseases undergoing corrective procedure with CPB were prospectively enrolled in this study. Circulating histones, N-terminal pro-brain natriuretic peptide (NT-proBNP), procalcitonin (PCT) and C-reactive protein (CRP) were measured preoperatively (T0) and at 0 (T1), 24 (T2), 48 (T3) and 72 (T4) h postoperatively. The relationships between biomarkers and clinical outcomes were analysed.

RESULTS: Circulating histones, NT-proBNP, PCT and CRP increased significantly postoperatively, with histones reaching the peak value earliest at T1. Circulating histone levels were higher in patients with adverse events. Receiver operating characteristic curve analysis showed that peak histone levels had a better predictive value for adverse events postoperatively. Peak histone levels correlated with the peak level of NT-proBNP ($r=0.563, P<0.01$), PCT ($r=0.551, P<0.01$), CRP ($r=0.606, P<0.01$) and clinical parameters such as ventilation time ($r=0.601, P<0.01$) and intensive care unit time ($r=0.623, P<0.01$).

CONCLUSIONS: Circulating histones reached peak levels faster than NT-proBNP, PCT and CRP. Furthermore, peak histone levels correlated with biomarkers and postoperative clinical outcomes. Circulating histones may be used as a prognostic indicator for patients after cardiac surgery with CPB.

CLINICAL TRIALS: ClinicalTrials.gov (ID: NCT02325765).

Keywords: Congenital heart disease • Circulating histones • Perioperative care • Surgery • Complications

INTRODUCTION

For patients with congenital heart diseases, cardiac surgery with cardiopulmonary bypass (CPB) is the classical treatment method. However, it is associated with a host of complications, including inflammation and heart failure [1]. Therefore, there is a need to develop biological markers that have the ability to predict the severity of illness and short-term clinical outcomes.

Histones are small, abundant proteins that are essential components of nucleosomes in eukaryotic cells. Circulating histones, either derived from apoptotic cells or secreted, are mediators of endothelial apoptosis, inflammation and organ failure [2, 3]. Circulating histones increase significantly after ischaemia-reperfusion injury and correlate with poor outcomes after trauma, thereby reflecting ongoing cellular damage or the inflammatory cytokine milieu [4].

Circulating histones in mice models are the major mediators of death and serve as a potential target in sepsis [2]. The circulating histone level is significantly higher in septic patients and correlate with disease severity [5]. It has been suggested that histones may be a biomarker for sepsis progression and prognosis.

We aimed to study the changes in circulating histone level, its correlation with clinical parameters and compare it with other biomarkers of prognosis in patients after cardiac surgery with CPB [N-terminal pro-brain natriuretic peptide (NT-proBNP), procalcitonin (PCT) and C-reactive protein (CRP)]. We hypothesized that patients with higher histone levels would have worse short-term clinical outcomes and circulating histones may guide the clinical management of patients.

MATERIALS AND METHODS

This study conformed to the principles outlined in the Declaration of Helsinki for the use of human blood and was approved by the Institutional Review Board of Shanghai Children’s Medical Center.
Affiliated to the Shanghai jiaotong University School of Medicine. And, all patients gave their written informed consent prior to their inclusion in this study.

Patients

The required sample size was calculated for comparison of the area under a receiver operating characteristic (ROC) curve with a null hypothesis value using the MedCalc software (version 12.0.0.0; MedCalc Software, Mariakerke, Belgium). No previous study has reported the predictive value of circulating histones for adverse events postoperatively; here 12 patients were enrolled in a preliminary study, and the area under the curve (AUC) was 0.88. The AUC was set to 0.7 to calculate the sample size, with a-level 0.05 and β-level 0.20 (power is 80%). The estimated total number of patients was 40. Estimating a dropout rate of 20%, 48 patients were asked to participate in this study.

A total of 48 consecutive patients (aged 1–36 months) with congenital heart diseases, who underwent corrective procedures with CPB between December 2014 and November 2015 at the Department of Cardiothoracic Surgery, Shanghai Children’s Medical Center, Shanghai jiao tong University School of Medicine, were prospectively included in our institutional database. Patients with any of the following were excluded from the study: weight less than 3 kg, previous palliative or corrective cardiac surgery, significant extra-cardiac anomalies or infection within 7 days before surgery.

Anaesthetic management

The patients were anaesthetized with midazolam (0.1 mg/kg), etomidate (0.3 mg/kg), sufentanil (2 µg/kg) and rocuronium (0.6 mg/kg) to facilitate tracheal intubation. Anaesthesia was maintained with an oxygen-air mixture containing sevoflurane (1–2%), sufentanil (25 µg/kg/h), rocuronium (0.5 mg/kg/h), propofol (4–6 mg/kg/h) and dexmedetomidine (0.5–1 µg/kg/h).

Surgical procedures

All procedures were performed through a median sternotomy with CPB and mild hypothermia (32–34°C). The aorta, superior vena cava and inferior vena cava were cannulated. Heparin was injected into the pump (20 mg) and the right atrial appendage (2 mg/kg) to maintain an activated clotting time above 400 s. Antegrade cold blood (20 ml/kg) was used to induce cardioplegia with additional blood injected (10 ml/kg) if the cross-clamp time exceeded 40 min. The pump flow was maintained at 100–150 ml/kg/min and the mean arterial pressure was maintained at 30–50 mmHg. Packed red blood cells were added to the CPB. Conventional and modified ultrafiltration was performed starting from the rewarming phase to 10–15 min after the cessation of CPB. After the surgery, intravenous protamine sulphate was administered to neutralize heparin.

Postoperative care

Patients were transferred to a paediatric cardiac intensive care unit (ICU) for postoperative care. All patients were monitored with invasive arterial and central venous pressure, electrocardiograms and rectal temperatures. Vasoactive drugs (dopamine, dobutamine, epinephrine, milrinone and norepinephrine) were administered to maintain the blood pressure. Mono- or bi-antimicrobial therapy was used according to the blood culture and inflammatory biomarkers.

Blood collection

Five arterial blood samples were collected in potassium–Ethylene Diamine Tetraacetic Acid tubes: preoperatively (T0), immediately after the operation (T1), 24 h postoperatively (T2), 48 h postoperatively (T3) and 72 h postoperatively (T4). After harvesting, blood samples were immediately centrifuged at 3000 rotations per minute (rpm) for 10 min at room temperature. The plasma was divided into small aliquots in polypropylene tubes and stored at −80°C. Frozen plasma samples were thawed once and immediately tested, with no repeated freezing and thawing.

Clinical parameters

The following data were recorded: CPB time, cross-clamp time, ventilation time, ICU time, hospital time, vasoactive-inotropic score, organ injury and other adverse events.

Vasoactive-inotropic score = dopamine dose (µg/kg/min) + dobutamine dose (µg/kg/min) +100 × epinephrine dose (µg/kg/min) + 10 × milrinone dose (µg/kg/min) + 10 000 × vasopressin dose (U/kg/min) + 100 × norepinephrine dose (µg/kg/min) [6].

Laboratory tests

Circulating histones were measured using a commercially available sandwich ELISA assay (Cell Death Detection ELISAplus kit: Roche Diagnostics, Indianapolis, IN, USA). The ELISA was developed and evaluated using 20 µl of sample (5 µl of plasma and 15 µl of incubation buffer) and 80 µl of immunoreagent per microplate (MP)-well; after incubation on an MP shaker with gentle shaking (300 rpm) for 2 h at +15 to +25°C, the solution was completely removed and each well was rinsed three times with incubation buffer. Then, each well was pipetted with 100 µl of ABTS solution and incubated on a plate shaker at 250 rpm until the colour development was sufficient for a photometric analysis. Each well was pipetted with 100 µl of ABTS Stop Solution. Finally, the samples were measured at 405 nm against ABTS solution +100 µl of ABTS Stop Solution as a blank (reference wavelength approx. 490 nm). All samples were analysed in duplicates according to instructions.

Serum PCT concentrations were measured by immunoluminometric assays with commercially available Elecsys BRAHMS PCT (Roche Diagnostics GmbH, Mannheim, Germany). CRP concentrations were measured by an immunoturbidimetric test (QuikRead go CRP, Diagnostica Oy, Espoo, Finland). NT-proBNP was measured with a commercially available immunochromatographic assay kit (Wuhan Easy Diagnosis Biomedicine Co., Ltd, Wuhan, China).

Data analysis

Statistical analyses were performed using SPSS version 22 (SPSS, Inc., Chicago, IL, USA), and the sample size was calculated by the Medcalc software. Data are presented as mean and standard
deviation, numbers (%) or median and interquartile range. Two-sided P-values <0.05 are considered to be statistically significant.

Linear regression analysis was used to evaluate the relationships of peak histone levels with peak levels of NT-proBNP, PCT, CRP and perioperative clinical parameters. Differences between the groups were investigated using Student’s t-test or the Mann-Whitney test, and a Pearson χ² test. Scatter diagrams were made using Prism 6 for Windows, version 6.01 (GraphPad Software, Inc., San Diego, CA, USA).

ROC curve and AUC were also calculated to evaluate the value of biomarkers in predicting adverse events postoperatively.

RESULTS

Patients and clinical parameters

The baseline characteristics of patients are listed in Table 1. Patients were divided into two groups according to the cut-off of histone level 0.582 μg/ml for predicting adverse events postoperatively using ROC curve analysis.

Forty-eight patients (54% males), with a median age of 7 months (1–24 months), were included in this study. Fifteen patients experienced adverse events postoperatively: pneumonia (n = 5), bi-antimicrobial therapy (n = 7), kidney injury (n = 2) and atrioventricular block (n = 1). All patients were alive at 6 months postoperatively.

None of the patients required inotropic drugs preoperatively. Patients with high histone levels required a higher dose and longer duration of inotropic support postoperatively (P = 0.01). However, pre- and postoperative left ventricular ejection fraction (LVEF) were similar between patients with high and low histone levels.

Adverse events postoperatively were higher in patients with histones above the cut-off than in those with histones below the cut-off (P < 0.01), with more incidences of pneumonia (P = 0.02) and bi-antimicrobial therapy (P = 0.04). Conversely, there were no difference in terms of kidney injury (P = 1) or atrioventricular block (P = 0.31). Patients with higher histone levels had significantly higher plasma NT-proBNP (P < 0.01), PCT (P = 0.01) and CRP (P < 0.01), compared with those with histones below the cut-off (0.582). Patients with high histone levels also had a significantly longer duration of ventilation support (P < 0.01), ICU stay (P < 0.01) and hospitalization (P < 0.01).

Serial temporal changes in histones and other biomarkers

The histone levels increased immediately postoperatively compared with at T0, reached the peak level at T1 and declined to baseline rapidly at T3 (Fig. 1A). As shown in Fig. 2, at T1 and T3, histone levels were higher in patients with adverse events compared with those without adverse events.

Table 1: Baseline characteristics and clinical parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients (n = 48)</th>
<th>Histones ≤0.582 (μg/ml) (n = 24)</th>
<th>Histones &gt;0.582 (μg/ml) (n = 24)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>7 (3, 12)</td>
<td>8.5 (4, 13)</td>
<td>6 (2, 10)</td>
<td>0.16</td>
</tr>
<tr>
<td>Male</td>
<td>26 (54%)</td>
<td>13 (54%)</td>
<td>13 (54%)</td>
<td>1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>6.7 ± 2.2</td>
<td>7.3 ± 2.4</td>
<td>6.1 ± 1.8</td>
<td>0.06</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>66.0 ± 9.5</td>
<td>68.9 ± 8.5</td>
<td>63.1 ± 9.7</td>
<td>0.21</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSD</td>
<td>16 (33%)</td>
<td>14 (58%)</td>
<td>2 (8%)</td>
<td>0.01</td>
</tr>
<tr>
<td>TOF</td>
<td>16 (33%)</td>
<td>7 (29%)</td>
<td>9 (38%)</td>
<td>0.54</td>
</tr>
<tr>
<td>TAPVC</td>
<td>8 (17%)</td>
<td>1 (4%)</td>
<td>7 (29%)</td>
<td>0.02</td>
</tr>
<tr>
<td>DORV</td>
<td>4 (8%)</td>
<td>1 (4%)</td>
<td>3 (13%)</td>
<td>0.61</td>
</tr>
<tr>
<td>TGA</td>
<td>4 (8%)</td>
<td>1 (4%)</td>
<td>3 (13%)</td>
<td>0.61</td>
</tr>
<tr>
<td>Aristotle score</td>
<td>8 (6, 9)</td>
<td>6 (6, 8)</td>
<td>9 (8, 10)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>7143.8 ± 2908.6</td>
<td>5700.0 ± 1836.1</td>
<td>8587.5 ± 3092.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PCT</td>
<td>3.0 ± 1.6</td>
<td>1.8 ± 0.8</td>
<td>4.2 ± 1.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CRP</td>
<td>31.3 ± 16.9</td>
<td>16.1 ± 8.7</td>
<td>37.4 ± 18.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>67.3 ± 25.8</td>
<td>50.9 ± 14.3</td>
<td>83.7 ± 24.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cross-clamp time (min)</td>
<td>42.2 ± 19.4</td>
<td>31.2 ± 12.5</td>
<td>53.3 ± 18.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ventilation time (h)</td>
<td>28 (17, 53)</td>
<td>22 (5, 28)</td>
<td>51 (29, 75)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ICU time (days)</td>
<td>4 (2, 6)</td>
<td>3 (1, 4)</td>
<td>5 (4, 7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hospital time (days)</td>
<td>12 (9, 15)</td>
<td>10 (8, 13)</td>
<td>14 (11, 18)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vasoactive-inotropic score</td>
<td>9.5 ± 3.5</td>
<td>7.7 ± 1.9</td>
<td>11.4 ± 3.8</td>
<td>0.01</td>
</tr>
<tr>
<td>LVEF pre-operation</td>
<td>71.4 ± 7.3</td>
<td>72.9 ± 8.1</td>
<td>70.0 ± 6.5</td>
<td>0.18</td>
</tr>
<tr>
<td>LVEF post-operation</td>
<td>67.6 ± 11.0</td>
<td>68.7 ± 9.5</td>
<td>66.5 ± 12.5</td>
<td>0.50</td>
</tr>
<tr>
<td>LVEF post-operation at 6 months</td>
<td>74.2 ± 7.9</td>
<td>72.0 ± 6.2</td>
<td>75.8 ± 8.8</td>
<td>0.14</td>
</tr>
<tr>
<td>Adverse events</td>
<td>15 (31%)</td>
<td>2 (8%)</td>
<td>13 (54%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>5 (10%)</td>
<td>0 (0%)</td>
<td>5 (21%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Bi-antimicrobial therapy</td>
<td>7 (15%)</td>
<td>1 (4%)</td>
<td>6 (25%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Kidney injury</td>
<td>2 (4%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>1</td>
</tr>
<tr>
<td>Atrioventricular block</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>1 (4%)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Data presented as mean and standard deviation, numbers (%) or median and interquartile range.

VSD: ventricular septal defect; TOF: tetralogy of Fallot; TAPVC: total anomalous pulmonary venous connection; DORV: double outlet right ventricular; TGA: transposition of the great arteries; NT-proBNP: N-terminal pro-brain natriuretic peptide; PCT: procalcitonin; CRP: C-reactive protein; CPB: cardiopulmonary bypass; ICU: intensive care unit; LVEF: left ventricular ejection fraction.
NT-proBNP levels increased significantly postoperatively at T1 compared with baseline, and peaked at T2. At T4, the levels decreased compared with T2 but remained higher than at T0 (Fig. 1B). PCT levels also increased postoperatively at T1 compared with baseline, peaked at T2 and then decreased, but remained higher than at T4 (Fig. 1C). There was no increase in CRP postoperatively at T1, but the peak CRP level at T3 was significantly higher than that at T0 (Fig. 1D).

The ROC curve analysis showed that peak histone levels could predict adverse events postoperatively with AUC = 0.814 (cut-off: 0.582 μg/ml; sensitivity: 87%; specificity: 73%), which was higher than that of NT-proBNP (AUC = 0.740; cut-off: 7500 pg/ml; sensitivity: 73%; specificity: 79%), PCT (AUC = 0.739; cut-off: 3.535 ng/ml; sensitivity: 53%; specificity: 84%) and CRP (AUC = 0.732; cut-off: 36.5 mg/l; sensitivity: 53%; specificity: 85%; Fig. 3).

Circulating histones had correlation with biomarkers and clinical parameters

On linear regression analysis, positive correlations were found between the peak histone levels and peak levels of NT-proBNP ($r = 0.563$, $P < 0.01$), PCT ($r = 0.551$, $P < 0.01$) and CRP ($r = 0.606$, $P < 0.01$) (Fig. 4). Peak histone levels also showed a significant correlation with ventilation time ($r = 0.601$, $P < 0.01$) and ICU time ($r = 0.623$, $P < 0.01$) (Fig. 5).
DISCUSSION

We conducted a single-centre study to evaluate the perioperative changes in histone levels and its prognostic value in 48 patients with congenital cardiac diseases undergoing a corrective procedure with CPB.

Circulating histones reached the peak level immediately after cardiac surgery at T1, which was earlier than NT-proBNP, PCT and CRP. This may be because histones are essential components of nucleosomes, are abundant in eukaryotic cells and are easily released into the blood by damaged or apoptotic cells. Severely injured tissues release large quantities of histones into the circulation [7]. In septic patients, circulating histones are derived from apoptotic cells or secreted and incorporated into neutrophil extracellular traps.

The functions of circulating histones are still unclear. Recent studies have demonstrated that high circulating histone levels are harmful and may cause endothelial damage, cytokine elevation, platelet aggregation, coagulation activation, thrombosis, lung injury, kidney injury, liver injury and even death [8–10]. In this study, patients with high histone levels had more adverse events with a greater incidence of pneumonia and bi-antimicrobial therapy. The peak histone levels had a moderate predictive power for adverse events postoperatively, with a cut-off of 0.582. Peak levels of PCT and CRP were higher in patients with histones above the cut-off than others. Peak histone levels were also correlated with peak PCT and CRP levels.

Figure 3: ROC analysis of histones, NT-proBNP, PCT and CRP for predicting adverse events. Histones (AUC = 0.814; cut-off: 0.582 μg/ml; sensitivity: 87%; specificity: 73%), NT-proBNP (AUC = 0.740; cut-off: 7500 pg/ml; sensitivity: 73%; specificity: 79%), PCT (AUC = 0.739; cut-off: 3.535 ng/ml; sensitivity: 53%; specificity: 84%) and CRP (AUC = 0.732; cut-off: 36.5 mg/l; sensitivity: 53%; specificity: 85%). ROC: receiver operating characteristic; AUC: area under the curve; NT-proBNP: N-terminal pro-brain natriuretic peptide; PCT: procalcitonin; CRP: C-reactive protein.

Figure 4: Correlation of peak histone levels with peak levels of biomarkers. (A) Correlation of peak histone levels with peak levels of NT-proBNP (r = 0.563, P < 0.01), (B) correlation of peak histone levels with peak levels of PCT (r = 0.551, P < 0.01) and (C) correlation of peak histone levels with peak levels of CRP (r = 0.606, P < 0.01). NT-proBNP: N-terminal pro-brain natriuretic peptide; PCT: procalcitonin; CRP: C-reactive protein.

Figure 5: Correlation between peak histone levels and clinical parameters. (A) Correlation between peak histone levels and ventilation time (r = 0.601, P < 0.01) and (B) correlation between peak histone levels and ICU time (r = 0.623, P < 0.01). ICU: intensive care unit.
PCT, one of the most effective markers of bacterial sepsis, is a pro-peptide of calcitonin produced by the thyroid gland [11]. It can be used for the early diagnosis and prognostic assessment of infections [12]. Patients with heart failure after cardiac surgery benefit from early detection of infections, which may be indicated by raised PCT [11].

CRP is an acute phase protein synthesized by the liver in response to infection, inflammation or tissue injury [13]. It is widely used as a systemic inflammatory response marker and a predictor of morbidity and mortality in cardiovascular diseases, including heart failure and after cardiac surgery [14]. However, CRP may also rise postoperatively and with some non-infectious inflammatory and viral diseases [5].

Early identification of infection has a major impact on the clinical course, antibiotic use and outcome of patients undergoing cardiac surgery with CPB. Early treatment improves prognosis, but routine use of antibiotics in all patients with systemic inflammatory response may lead to the selection of resistant strains, and increased toxicity and cost [15].

Circulating histones damage endothelial cells by integrating into the cell membrane, increasing cell membrane permeability and causing an influx of calcium [16]. In vitro exposure of cardiomyocytes to histones damages calcium and redox system homeostasis and mitochondrial function. Perfusion of hearts with histones also causes electrical and functional dysfunction. In vivo neutralization of histones in septic mice markedly reduces heart dysfunction [17]. Histones also play a role in heart failure and myocardial remodelling after ischaemia–reperfusion injury [18].

In this study, patients with high histone levels needed a higher dose and longer duration of inotropic support. Previous studies have shown that patients requiring greater inotropic support after cardiac surgery with CPB had higher morbidity and mortality. A high vasoactive-inotropic score in the first 48 h postoperatively was a surrogate marker of heart function and illness severity [6].

Circulating histones reached a peak level immediately after the surgery, which was earlier than NT-proBNP. A higher peak histone level was also associated with higher peak NT-proBNP level. NT-proBNP is a cardiac hormone with diuretic, natriuretic and vasodilator properties. It is secreted mainly by the ventricles in response to volume expansion and pressure load. It is an effective marker of severity and prognosis of incident cardiovascular events and heart failure after congenital heart surgery [19, 20].

Previous studies have shown that NT-proBNP reaches its highest concentration 24 h after cardiac surgery. Higher peak levels of NT-proBNP are associated with longer duration of CPB, aortic cross-clamp time and ICU stay [21].

The Aristotle score ranks the complexity of 145 congenital cardiac surgery procedures [22]. Our study has shown that patients with high histone levels had higher Aristotle scores, meaning that these patients underwent more complex surgical procedures (such as total anomalous pulmonary venous connection).

Patients with histones above the cut-off had a longer duration of intraoperative CPB, cross-clamp time, ventilation, hospitalization and ICU admission than those with histones below the cut-off. CPB duration is a potent stimulus of the systemic inflammatory response. Young patients have a more severe inflammatory response to CPB because of higher metabolic demands, reactive pulmonary vasculature, immature organ systems, altered homoeostasis and a high ratio of extracorporeal circuit length to patient size [23]. Numerous studies have shown activation of the complement cascade, release of endotoxins and altered production of cytokines during and after cardiac surgery [24].

There was no difference in the LVEF measured preoperatively and postoperatively, probably because several factors influence heart output such as heart structure, inotropic drugs, inflammation and organ dysfunction. Therefore, the LVEF cannot be used as a marker of heart function and illness severity in patients after cardiac surgery.

In young patients undergoing cardiac surgery, accurate risk prediction is vital for clinical management, benchmarking and identifying high-risk patients who may benefit from prophylactic interventions. The major limitation of this study was a small sample size, without severe illnesses. Further studies with a larger sample size and wider variety of congenital heart diseases are warranted to provide more reliable results.

**CONCLUSION**

Circulating histones reached peak level immediately after cardiac surgery in patients and earlier than NT-proBNP, PCT and CRP. Peak histone levels positively correlated with other biomarkers and clinical outcomes, and had a moderate predictive value for adverse events. These findings suggest that circulating histones may be used to predict adverse events after cardiac surgery and thereby improve the management of patients.

**ACKNOWLEDGEMENTS**

The authors are grateful to the cardiac surgeons, anaesthesiologists and nurses at the Department of Cardiothoracic Surgery, Shanghai Children’s Medical Center for their help with this study.

**Conflict of interest:** none declared.

**REFERENCES**


