The combined use of neutrophil gelatinase-associated lipocalin and brain natriuretic peptide improves risk stratification in pediatric cardiac surgery

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Abstract

Background: The aim of this study is to test the hypothesis whether the combined use of a cardio-specific biomarker, the brain natriuretic peptide (BNP), and a marker of early renal damage, the assay of urinary neutrophil gelatinase-associated lipocalin (uNGAL), may improve risk stratification in pediatric cardiac surgery.

Methods: We prospectively enrolled 135 children [median age 7 (interquartile range 1–49) months] undergoing to cardiac surgery for congenital heart disease. All biomarkers were evaluated pre- and post-operatively at different times after cardiopulmonary-bypass (CPB): uNGAL at 2, 6 and 12 h; BNP at 12 and 36 h; serum creatinine at 2, 6, 12, and 36 h. Primary endpoints were development of acute kidney injury (AKI) (defined as 1.5 serum creatinine increase) and intubation time.

Results: AKI occurred in 39% of patients (65% neonates and 32% older children, p=0.004). The peak of uNGAL values occurred more frequently at 2 h. uNGAL values at 2 h [median 28.2 (interquartile range 7.0–124.6) ng/L] had a good diagnostic accuracy for early diagnosis of AKI with an AUC [median 28.2 (interquartile range 7.0 – 124.6) ng/L] had a good diagnostic accuracy for early diagnosis of AKI with an AUC (area under the curve) ROC (receiver operating characteristic) curve of 0.85 (SE 0.034). Using multivariable logistic regression analysis, development of AKI was significantly associated with uNGAL values at 2 h after CPB [OR=1.88 (1.30–2.72, p=0.001)], together with the CPB time and Aristotle score, as an index of complexity of the surgical procedure, while pre-operative BNP values were not. Furthermore, uNGAL and pre-operative BNP values (together with Aristotle score) were significantly associated with adverse outcome (longer intubation time and mortality).

Conclusions: Pre-operative BNP and uNGAL values after surgery (together with the Aristotle score) were independently associated with a more severe course and worse outcome in children undergoing cardiac surgery for congenital heart disease.

Keywords: brain natriuretic peptide (BNP); children; neutrophil gelatinase-associated lipocalin (NGAL); pediatric cardiac surgery; renal damage.

Introduction

Acute kidney injury (AKI) is a common and severe complication in children undergoing cardiac surgery for congenital heart disease (1–9), with important consequences in terms of cost, morbidity and mortality. Unfortunately, diagnosis and management of AKI in this clinical setting are at present inadequate (10–14). This is at least partly due to the incomplete understanding of the pathophysiology of renal damage after pediatric cardiac surgery, which limits diagnostic and therapeutic approaches. Recently, AKI syndromes, including those complicating cardiac surgery, are considered to be a consequence of complex interactions between cardiac and renal injury, so called cardiorenal syndromes (10–12).

Previous studies on cardiorenal syndromes were focused on the renal ischemia-reperfusion damage caused by the systemic inflammatory response caused by cardio-pulmonary bypass, the hemodynamic instability after surgery and/or the use of potentially nephrotoxic drugs (such as loop diuretics) (10, 11). Although a diseased heart has several negative effects on kidney function, at the same time, renal failure can significantly affect cardiac function (12). Thus, direct and indirect effects of heart or kidney dysfunction can thus, initiate and perpetuate the disorder of both organs through a complex combination of neurohormonal feedback mechanisms (12).

The interest in biomarkers of cardiac and renal damage in children undergoing cardiac surgery for congenital heart disease has progressively increased during the last few years (1–9). The clinical relevance of brain natriuretic peptide (BNP) in pediatric cardiac surgery has been acknowledged in the last few years (1), although extensive studies are still lacking. The clinical relevance of novel biomarkers more specific for AKI syndromes, however, have been recently evaluated in adult and pediatric cardiac surgery (4–9), including the neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1) and liver-type fatty acid binding protein (L-FABP) (3, 12).
The diagnosis of AKI is currently based on serial serum creatinine measurements, which present some limitations leading to a consistent delay in the diagnostic process of AKI and failure in the identification of sub-clinical damage (2, 3, 15–18).

It is theoretically conceivable that the use of a biomarker specific for the renal tubular damage may improve the diagnosis and management of AKI syndromes (3, 18). Among the novel new biomarkers recently suggested for AKI syndromes, the recent guidelines from the Acute Dialysis Quality Initiative (ADQI) (17) reported that NGAL could be integrated into clinical practice in the near future. The few studies conducted in patients with congenital heart defects undergoing cardiac surgery, however, reported conflicting results (4–9). In particular, large differences in diagnostic accuracy of NGAL in detecting AKI were found (18, 19). These discrepancies between studies may be attributable to differences in experimental protocols (such as the choice of different sampling times or clinical conditions of the enrolled patients) and criteria adopted for the diagnosis of AKI (15, 20). None of the previous studies moreover, on AKI after pediatric cardiac surgery (4–9) investigated the cardiorenal syndrome as a whole, only evaluating the clinical performance of some biomarkers of renal damage.

The main aim of the present study is to test the hypothesis whether the combined use of a cardio-specific biomarker, the BNP and a marker of early renal damage, the assay of urinary neutrophil gelatinase-associated lipocalin (uNGAL), may improve risk stratification in pediatric cardiac surgery. Another aim of this study is to evaluate whether the measurement of uNGAL and plasma BNP may significantly and independently improve the diagnostic accuracy of AKI in these patients.

Materials and methods

Experimental protocol

Between December 2010 and November 2011, we prospectively enrolled 135 children undergoing cardiac surgery for correction/palliation of congenital heart defects at the Pediatric Cardiac Surgery Division, Heart Hospital, Fondazione G. Monasterio, Massa, Italy. Patients were excluded if they had a history of prior renal transplantation or dialysis requirements.

The study was approved by the Local Ethic Committee. Informed consent was obtained from parents of all the children enrolled in the study.

Biomarker measurements

Laboratory investigators were blinded to clinical outcomes. The concentrations of NGAL in urine specimens (uNGAL) were measured by a fully automated immunoassay using the ARCHITECT platform (Abbott Diagnostics Laboratories, Abbott Park, IL, USA). Urine samples were collected before and at 2, 6 and 12 h after termination of cardiopulmonary bypass and stored in aliquots at −80°C. Because creatinine was also measured in the same urine sample, it was possible to calculate the ratio between the NGAL and creatinine concentrations.

Creatinine in serum and urine samples was measured by an enzymatic method (21) with the UniCel DxC 600 platform (CR-E, REF A60298, Beckam Coulter Beckman Coulter, Inc., Fullerton, CA, USA). Blood and urine samples for the measurement of creatinine were collected pre-operatively and also post-operatively at 2, 6, 12, and 36 h after termination of cardio-pulmonary bypass (CPB). Urine samples were also collected on the ward until hospital discharge, dependent upon the patients’ clinical condition and treatment.

Blood samples for BNP measurement were collected pre-operatively and then at 12 h after operation, using only blood samples taken for clinical necessity (at 7:30 am). No additional samples were withdrawn. Plasma BNP was measured using the fully automated Access platform (Triage BNP reagents, Access Immunoassay Systems, REF 98200; Beckman Coulter, Inc., Fullerton, CA, USA). The analytical characteristics and performance of the Access Immunoassay method used in this study for measurement of BNP had been previously evaluated in our laboratory (22, 23).

Arterial blood gases and arterial lactate were measured with a fully automated assay (ABL 700 series Radiometer Copenhagen) at the same points of those of serum creatinine.

Outcome and variables definitions

The primary outcomes of the study were the presence of an AKI syndrome and the intubation time after cardiac surgery. The criteria for the presence of AKI were an increase of 1.5-fold or greater of plasma creatinine from pre-operative baseline levels according to the RIFLE classification modified for pediatric patients (15, 16). Surgical procedures were classified according to the Aristotle score, as index of complexity of surgical procedure (24). Clinical parameters, including age, gender, weight, height, body surface area, and duration of cardiopulmonary bypass were collected for every patient. Adverse events were recorded up to 30 days after cardiac surgery according to Portman et al. (25).

Surgical and clinical management

The pre-operative anesthesia management, intra-operative bypass strategy, and subsequent management in the intensive care unit (ICU) followed standard institutional practice. Non-iodinated topical antiseptics were used for every patient. A standard technique was used to institute CPB (roller pump, disposable membrane oxygenator and arterial filter) and involved bivacal drainage and ascending aorta perfusion. Different myocardial protection (anterograde cold crystalloid cardioplegia or with cold blood cardioplegia) and degrees of body temperature were used (ranging from 35°C to 19°C) depending on the surgical strategy. In the post-operative period, hemodynamic management was conducted using epinephrine (0.005–0.15 ng/kg/min), milrinone (0.5–0.75 ng/kg/min), dopamine (5–20 ng/kg/min), and noradrenaline (0.05–0.5 ng/kg/min). Intravascular volume expansion was conducted according to the attending physician and consisted of 20% human albumin or fresh-frozen plasma. Diuretics usually consisted of furosemide (1–10 mg/kg/day). Echocardiograms were performed before and after cardiac surgery (usually between 12 and 24 h after operation) and ejection fraction was derived according to current guidelines (26).

Statistical analysis

Data were expressed in term of median (25th–75th percentiles) for continuous variables and number of subjects (percentage) for categorical variables. Comparison between groups were performed by
Fisher test, χ²-test, independent Student’s t-test and non-parametric Mann-Whitney U-test. Spearman’s rho was used to analyze the relation between variables.

Biomarker values over time were evaluated with mixed-effects regression models (MRMs) to properly account for correlation among repeated measures and missing values. Receiver operating characteristic (ROC) curves and the area under the curve (AUC) were used to assess the discriminatory ability of uNGAL. Univariable and multivariable logistic regression was used to identify variables associated with AKI. The intubation time (or extubation time, TTE) was studied in term of time to event variable. To do this, zero time was considered as the time of intubation (tube placement) and extubation (tube removal) events were considered until the 15th day post-surgery. Deceased patients or those requiring longer than 15 days intubation were censored. The Kaplan-Meier method and log rank test were used to compare the TTE values across groups. Cox proportional-hazard models were used to identify variables affecting the TTE. Due to the fact we considered TTE as the event studied in term of time to event analysis, hazard ratio (HR) should be interpreted as the indicator of chance to extubation with HR lower than one indicating a low risk of TTE (negative outcome) and HR higher than one suggesting a higher chance of extubation (positive outcome). For both logistic regression and Cox proportional-hazard model variables with p < 0.1 at univariable analysis were considered for multivariable models. Proportional hazard assumption was checked using the Schoenfeld test and no significant departures from this assumption were observed. Logarithmic transformation was used for variables which are not normally distributed. A 2-tailed value of p < 0.05 was considered statistically significant. SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) was used for analysis.

### Results

#### Clinical data

Clinical and demographic characteristics as well as the results of laboratory tests of the enrolled patients in basal conditions (i.e., pre-operatively) are reported in Table 1, while the parameters of outcome divided according to the different age-subgroups in Table 2.

AKI occurred in 52 children (39%). In particular, AKI occurred in 65% of neonates vs. 32% of older children (p = 0.004). On average, clinical conditions of neonates were more severe than those of older children, as demonstrated by a higher operative risk, expressed by the Aristotle score (p = 0.028), and a post-operative period characterized by a longer median intubation time (p < 0.001), length of stay in the ICU (p < 0.001) and need for inotropic support (p = 0.011).

### Table 1 Patients characteristics.

<table>
<thead>
<tr>
<th>Characteristics, units</th>
<th>AKI (n=52)</th>
<th>Non AKI (n=83)</th>
<th>Total (n=135)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, months</td>
<td>3 (0–33)</td>
<td>11 (4–60)</td>
<td>7 (1–49)</td>
</tr>
<tr>
<td>Male, %</td>
<td>30 (57.7)</td>
<td>48 (57.8)</td>
<td>78 (57.8)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>4.8 (3.4–12.3)</td>
<td>8.6 (4.9–17.0)</td>
<td>7 (3.8–13.4)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>57 (51.5–89.5)</td>
<td>72.5 (60.0–109.8)</td>
<td>67.5 (55.0–98.8)</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>5.1 (4.8–6.1)</td>
<td>5.6 (5.2–6.7)</td>
<td>5.5 (5.0–6.4)</td>
</tr>
<tr>
<td>Glenn, %</td>
<td>2 (3.85)</td>
<td>1 (1.20)</td>
<td>3 (2.24)</td>
</tr>
<tr>
<td>LRVO, %</td>
<td>–</td>
<td>4 (4.82)</td>
<td>4 (2.99)</td>
</tr>
<tr>
<td>Left ventricular pressure overload, %</td>
<td>8 (15.38)</td>
<td>9 (10.84)</td>
<td>17 (12.69)</td>
</tr>
<tr>
<td>Left ventricular volume overload, %</td>
<td>16 (30.77)</td>
<td>25 (30.12)</td>
<td>41 (30.6)</td>
</tr>
<tr>
<td>Palliated UH, %</td>
<td>2 (3.85)</td>
<td>4 (4.82)</td>
<td>6 (4.48)</td>
</tr>
<tr>
<td>Right ventricular pressure overload, %</td>
<td>12 (23.08)</td>
<td>12 (14.46)</td>
<td>24 (17.91)</td>
</tr>
<tr>
<td>Right ventricular volume overload, %</td>
<td>2 (3.85)</td>
<td>19 (22.89)</td>
<td>21 (15.67)</td>
</tr>
<tr>
<td>Transposition of the great arteries, %</td>
<td>5 (9.62)</td>
<td>3 (3.61)</td>
<td>8 (5.97)</td>
</tr>
<tr>
<td>Univentricular heart, %</td>
<td>5 (9.62)</td>
<td>6 (7.23)</td>
<td>11 (8.21)</td>
</tr>
<tr>
<td>BNP, ng/L</td>
<td>149 (54.5–938.0)</td>
<td>57 (35.0–116.8)</td>
<td>86 (40.8–216.8)</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.24 (0.19–0.30)</td>
<td>0.24 (0.18–0.30)</td>
<td>0.24 (0.18–0.30)</td>
</tr>
<tr>
<td>Urinary creatinine, mg/dL</td>
<td>23.20 (7.55–54.88)</td>
<td>3.80 (20.55–91.70)</td>
<td>46.55 (16.09–78.20)</td>
</tr>
<tr>
<td>u-NGAL, ng/mL</td>
<td>7.65 (3.55–27.18)</td>
<td>8.30 (3.70–20.95)</td>
<td>8.30 (3.7–21.5)</td>
</tr>
<tr>
<td>u-NGAL/urinary creatinine ratio</td>
<td>4.30 (1.60–9.35)</td>
<td>2.20 (0.63–6.18)</td>
<td>2.80 (0.75–7.03)</td>
</tr>
<tr>
<td>CPB time, min</td>
<td>153 (120–201.3)</td>
<td>98 (65–115)</td>
<td>112 (76–150)</td>
</tr>
<tr>
<td>Cross-clamp, min</td>
<td>83 (29–122)</td>
<td>47 (27–67)</td>
<td>58 (28–83)</td>
</tr>
<tr>
<td>Intubation time, h</td>
<td>48 (12–111)</td>
<td>8 (5–14)</td>
<td>12 (5–61)</td>
</tr>
<tr>
<td>Inotropic time, h</td>
<td>108 (36–182)</td>
<td>12 (10–60)</td>
<td>36 (12–108)</td>
</tr>
<tr>
<td>ICU stay, h</td>
<td>108 (36–156)</td>
<td>24 (12–36)</td>
<td>36 (12–108)</td>
</tr>
<tr>
<td>Aristotle score</td>
<td>8.37 (2.00)</td>
<td>6.58 (2.18)</td>
<td>7.27 (2.28)</td>
</tr>
</tbody>
</table>

*p < 0.05 Mann-Whitney U-test or independent Student’s t-test. Congenital heart diseases have been divided into main groups according to the hemodynamic (23): Biventricular volume overload group (atrio-ventricular defects); Left ventricular pressure overload group (including aortic stenosis and aortic coarctation); Left ventricular volume overload group (ventricular septal defects, significant patent arterial duct, truncus arteriosus); Right ventricular pressure overload group (tetralogy of Fallot, pulmonary stenosis); right ventricular volume overload group (atrial septal defect, anomalous pulmonary venous drainage). The conversion factor for creatinine from mg/dL to SI Units is 88.4. The uNGAL/urinary creatinine ratio was calculated by the formula: ratio value = (uNGAL, expressed as ng/mL or μg/L)/(urinary creatinine, expressed as mg/dL)×10."
Table 2 Parameters of outcomes divided according to the different age-subgroups.

<table>
<thead>
<tr>
<th>Parameters, units</th>
<th>Neonates (n=26)</th>
<th>Older children (n=109)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPB time, min</td>
<td>137 (110–170)</td>
<td>107 (74–143)</td>
<td>0.071</td>
</tr>
<tr>
<td>Cross-clamp, min</td>
<td>49 (6–87)</td>
<td>61 (30–84)</td>
<td>0.236</td>
</tr>
<tr>
<td>Intubation time, h</td>
<td>84 (15–174)</td>
<td>8 (5–18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inotropic time, h</td>
<td>108 (33–228)</td>
<td>12 (10–84)</td>
<td>0.011</td>
</tr>
<tr>
<td>ICU stay, h</td>
<td>108 (42–225)</td>
<td>36 (12–60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aristotle score</td>
<td>8.16 (2.68)</td>
<td>7.06 (2.13)</td>
<td>0.028</td>
</tr>
<tr>
<td>Adverse events, %</td>
<td>5 (19.2)</td>
<td>9 (8.3)</td>
<td>0.145</td>
</tr>
<tr>
<td>[Type of events (n)]</td>
<td>[LCO (2), Redo (2), Death (1)]</td>
<td>[LCO (6), Redo (1), CA (2)]</td>
<td></td>
</tr>
</tbody>
</table>

CA, cardiac arrest; ICU, intensive care unit; LCO, low cardiac output; Redo, patients requiring re-operation.

Due to the more severe clinical conditions as well as the higher number of neonates, the group of patients with AKI showed also significantly higher (p=0.001) values of BNP than patients without AKI (Table 1).

NGAL and BNP levels after cardiac surgery

Considering all patients as a whole group, uNGAL values significantly increased post-surgery, with a peak [40.1 (8.0–156.4) ng/mL] usually occurring between 2 and 6 h, being more frequent at 2 h (corresponding to the 64% of the total number of cases). In particular, uNGAL values at 2 h [28.2 (7.0–124.6) ng/mL] were significantly higher than in the basal condition [8.3 (3.7–21.5) ng/mL; p<0.001]). After this initial, early increase a progressive decrease of uNGAL was observed at 6 and 12 h [18.4 (4.1–71.6) ng/mL and 3.8 (1.8–16.6) ng/mL, respectively]. It is important to note that the time-courses of uNGAL values were different in patients with and without AKI. Indeed, in patients with AKI the uNGAL values at 2 h were significantly higher than basal values (Figure 1, part A.2), while in patients without AKI these uNGAL values were not significantly different to basal ones (Figure 1, part A.1).

According to the diagnosis of AKI (15, 16), serum creatinine values significantly increased only in patients with AKI (Figure 1, part B.2). Serum creatinine values progressively increased after surgery, reaching the peak at 36–60 h.

![Figure 1](image-url)
in patients with AKI; as a result, the concentration peak of uNGAL occurred significantly earlier than that of serum creatinine (p<0.001) (Figure 1, part A.2 and part B.2).

Urinary creatinine showed a fall after surgery (p<0.001) and an increase later with a peak at 12–36 h in both patients with and without AKI (Figure 2). The ratio between the concentration of NGAL and creatinine in urine samples significantly increased after surgery (p<0.001), peaking at 2-h and decreasing subsequently with a time course similar to that of uNGAL (Figure 2).

Considering all patients as a whole, BNP values increased after surgery [from 86 (40.8–216.8) ng/L before surgery to 428.5 (271.0–891.0) ng/L at 12 h after surgery; p<0.001], showing similar time-courses in patients with or without AKI.

Diagnostic accuracy of NGAL in the early diagnosis of AKI

uNGAL at 2 h showed a good diagnostic accuracy for the diagnosis of AKI, with an AUC of 0.85 (SE 0.034) with a cut-off value of 49.95 ng/mL, a sensitivity 0.784 and a specificity of 0.815. Data concerning the analysis of ROC curves, corresponding to samples, collected at different times throughout the study, are reported in Table 3.

A significant diagnostic accuracy for the presence of AKI was also found for BNP levels 12 h after surgery, with an AUC of 0.70 (sensitivity 0.712 and specificity 0.612) with a best cut-off value of 423.5 ng/L.

Inter-relationships between biomarker values and clinical outcomes

A significant negative correlation was found between uNGAL values and body surface area (BSA) (ρ=−0.23, p=0.031). All uNGAL values observed after surgery positively correlated with the severity of the cardiac disease (ρ=0.26, p=0.004 for uNGAL 2 h post-surgery), as assessed by pre-surgery BNP values, as well as with the indicators of surgery complexity, as assessed by the Aristotle score (ρ=0.28, p=0.001 for uNGAL 2 h post-surgery) and CPB-time (ρ=0.62, p<0.001 for uNGAL 2 h post-surgery). Highly significant positive correlations were also found between uNGAL values after surgery and parameters of outcomes, such as intubation duration (p=0.43, p<0.001 for uNGAL 2 h post-surgery) and time spent in the ICU (p=0.46, p<0.001 for uNGAL 2 h post-surgery).

Pre-surgery BNP values correlated negatively with BSA and age (ρ=−0.56, p<0.001 and ρ=−0.51, p<0.001, respectively), and positively with intubation duration (ρ=0.44, p<0.001),
Table 3  Data of ROC analysis. The values of best cut-off value, AUC, sensitivity and specificity (calculated at the best cut-off) are reported.

<table>
<thead>
<tr>
<th>Data group</th>
<th>uNGAL, ng/mL</th>
<th>AUC (SE)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h post-surgery</td>
<td>49.95</td>
<td>0.85 (0.034)</td>
<td>0.784</td>
<td>0.815</td>
</tr>
<tr>
<td>6 h post-surgery</td>
<td>22.00</td>
<td>0.85 (0.036)</td>
<td>0.813</td>
<td>0.734</td>
</tr>
<tr>
<td>12 h post-surgery</td>
<td>3.55</td>
<td>0.78 (0.042)</td>
<td>0.766</td>
<td>0.610</td>
</tr>
<tr>
<td>Peak among 12 h post-surgery</td>
<td>64.75</td>
<td>0.87 (0.036)</td>
<td>0.824</td>
<td>0.802</td>
</tr>
</tbody>
</table>

Peak within 12 h after surgery: the highest value measured within 12 h after surgery.

length of stay in the ICU (p=0.42, p<0.001), and Aristotle score (p=0.24, p=0.007). BNP values at 12 h after surgery were also significantly correlated with 2 h uNGAL (p=0.321, p<0.001), thus suggesting a link between cardiovascular and renal syndrome. Furthermore, BNP values at 12 h after surgery correlated with arterial lactate (p=0.44, p<0.001), left ventricular ejection fraction (p=-0.30, p=0.007) and arterial blood pH (p=0.40, p=0.002) at 12 h after surgery.

Logistic regression

Using a univariable logistic regression, all uNGAL and NGAL/creatinine ratio values after surgery were found to be significantly associated with diagnosis of AKI, differently from the pre-operative values. The univariable analysis also indicated that BSA, Aristotle score, CPB time, and BNP were significantly associated with the diagnosis of AKI (Table 4). Using a multivariate analysis, the uNGAL values at 2 h post-surgery resulted independently associated with diagnosis of AKI, together with the CPB time and the Aristotle score (Table 4).

Univariable and multivariable Cox regression models

Univariable Cox models showed that higher Aristotle score and CPB time, but lower age and BSA, were significantly associated with an increased risk of longer intubation times (Table 5). Lower pre-surgery urinary creatinine, higher pre-operative and 12-h BNP and higher 2-h and peak uNGAL values moreover, were significant predictor of longer intubation times. At multivariable analysis, Aristotle score, basal BNP and uNGAL at 2 h post-surgery remained the only significant predictors of intubation time (Table 5).

Discussion

The principal aim of the present study was to evaluate whether the combined use of a cardio-specific biomarker, such as BNP (27), associated with a marker of early renal damage, such as uNGAL (3, 12), may provide a better risk stratification in pediatric patients with congenital heart defects undergoing cardiac surgery. Another aim of this study was to evaluate whether the measurement of uNGAL and plasma BNP may significantly and independently improve the diagnostic accuracy of AKI in these patients.

As far as the risk stratification is concerned, the results of the present study demonstrate that uNGAL is strongly and independently associated with some adverse events in pediatric patients with congenital heart defects undergoing cardiac surgery (including the longer intubation time and the period of time spent in the ICU) (Table 5). Our study also indicates that the pre-operative BNP values were independently associated with a more severe outcome in pediatric patients undergoing cardiac surgery. The results of our study therefore indicate for the first time that the combined use of uNGAL and BNP can improve risk stratification in pediatric patients with congenital heart defects undergoing cardiac surgery.
The development of renal complications after cardiac surgery portends significant morbidity and mortality (33). In particular, depending on the definition, post-operative AKI occurs in 3%–30% of patients, with the need of renal replacement therapy in 1%–5% (33). The prognosis among this subgroup of patients is poor, with an increased mortality risk exceeding 60% compared with the overall mortality rate of 2%–8% after cardiac surgery (33). uNGAL after surgery moreover, (but not BNP) is a significant and independent predictor for developing AKI. The measurement of uNGAL alone in particular, was able to allow a correct diagnosis of AKI in about 80% of patients at least 1 day before that of serum creatinine. Clinicians could use this gain in time to initiate an earlier and more appropriate treatment.

**Conclusions**

The development of renal complications after cardiac surgery portends significant morbidity and mortality (33). In particular, depending on the definition, post-operative AKI occurs in 3%–30% of patients, with the need of renal replacement therapy in 1%–5% (33). The prognosis among this subgroup of patients is poor, with an increased mortality risk exceeding 60% compared with the overall mortality rate of 2%–8% after cardiac surgery (33). uNGAL after surgery moreover, (but not BNP) is a significant and independent predictor for developing AKI. The measurement of uNGAL alone in particular, was able to allow a correct diagnosis of AKI in about 80% of patients at least 1 day before that of serum creatinine. Clinicians could use this gain in time to initiate an earlier and more appropriate treatment.

**Conflict of interest statement**

Authors’ conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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Employment or leadership: None declared.

Honourarium: None declared.

References

1. Cantinotti M, Giovannini S, Murzi B, Clerico A. Diagnostic, 
prognostic and therapeutic relevance of B-type natriuretic peptide 
assay in children with congenital heart diseases [review]. 

2. Haase M, Devajaran P, Haase-Fielitz A, Bellomo R, Cruz DN, 
Wagener G, et al. The outcome of neutrophil gelatinase-associated 
lipocalin-positive subclinical acute kidney injury: a multi-
center pooled analysis of prospective studies. J Am Coll Cardiol 

3. Devajaran P. Biomarkers for the early detection of acute kidney 

injury and poor outcomes after paediatric cardiac surgery. J Am Soc 

5. Bennett M, Dent CL, Ma Q, Dastrala S, Grenier F, Workman 
R, et al. Urine NGAL predicts severity of acute kidney injury 

P. Neutrophil gelatinase-associated lipocalin concentrations 
predict development of acute kidney injury in neonates and children 

7. Dent CL, Ma Q, Dastrala S, Bennett M, Mitsnedges MM, Barasch 
acute kidney injury, morbidity and mortality after paediatric 
cardiac surgery: a prospective uncontrolled cohort study. Crit Care 
2007;11:R127.

M. Plasma neutrophil gelatinase-associated lipocalin measured 
in consecutive patients after congenital heart surgery using 
point-of-care technology. Interact CardioVasc Thorac Surg 

Ma Q, et al. Temporal relationship and predictive value of urinary 
acute kidney injury biomarkers after paediatric cardiopulmonary 

10. Price JF, Goldstein SL. Cardiorenal syndrome in children with 

11. Price JF, Mott AR, Dickerson HA, Jefferies JL, Nelson DP, 
Chang AC, et al. Worsening renal function in children hospital-
ized for decompensated heart failure: evidence for a paediatric 


Schwartz D. Anemia, chronic renal disease and congestive heart 
failure – the cardio renal anaemia syndrome: the need for coopera-
tion between cardiologists and nephrologists. Int Urol Nephrol 

15. Akcan-Arikan A, Zappitelli M, Loftis LL, Washburn KK, 
Jefferson LS, Goldstein SL. Modified RIFLE criteria in critically 
ill children with acute kidney injury. Kidney Int 2007;71: 
1028–35.

16. Zappitelli M, Parikh CR, Akcan-Arikan A, Washburn KK, 
Moffett BS, Goldstein SL. Ascertainment and epidemiology of 
acute kidney injury varies with definition interpretation. Clin J 

17. Ronco C, Mccullough P, Anker SD, Anand I, Aspromonte N, 
Bagshaw SM, et al. Cardio-renal syndromes: report from the 
consensus conference of the Acute Dialysis Quality Initiative. 
Eur Heart J 2010;31:703–11.

18. Clerico A, Galli C, Fortunato A, Ronco C. Neutrophil gelatinase-
associated lipocalin (NGAL) as biomarker of acute kidney injury: 
a review of the laboratory characteristics and clinical evidences. 

Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin 
(NGAL) in diagnosis and prognosis in acute kidney injury: a 
 systematic review and meta-analysis. Am J Kidney Dis 2009:54: 
1012–24.

outcomes from acute kidney injury: report of an initiative. Pediatr 

colorimetric method based on hydrogen peroxide measurement. 

22. Prontera C, Storti S, Passino C, Zyw L, Zucchelli 
GC, et al. Comparison of a fully automated immunoassay with 
a point-of-care testing method for B-type natriuretic peptide. 

23. Cantinotti M, Storti S, Passino C, Clerico A. Clinical relevance 
of time course of BNP levels in neonates with congenital heart 

24. Lacour-Gayet F, Clarke D, Jacobs J, Comas J, Daebritz S, 
method to evaluate surgical results. Eur J Cardithorac Surg 

25. Portman MA, Skee A, Olson AK, Cohen G, Karl T, Tong E, 
et al. Triiodothyronine supplementation in infants and children 
undergoing cardiopulmonary bypass (TRICC): a multicenter 
placebo-controlled randomized trial: age analysis. Circulation 
2010;14:S224–33.

26. Lopez L, Colan SD, Frommetl PC, Ensing GJ, Kendall K, 
Younoszai AK, et al. Recommendations for quantification 
methods during the performance of a paediatric echocardiogram: 
a report from the paediatric measurements writing group of the 
American Society of Echocardiography Paediatric and 
Congenital Heart Disease Council. J Am Soc Echocardiogr 

27. Clerico A. Pathophysiological and clinical relevance of circulat-
ing levels of cardiac natriuretic hormones: are they merely mark-

Wagener G, et al. The outcome of neutrophil gelatinase-associ-
ated lipocalin-positive subclinical acute kidney injury: a multis-

on-pump coronary artery surgery: focus on inflammation,