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

Massimiliano Cantinotti[1], Simona Storti[1], Valentina Lorenzoni[2], Luigi Arcieri[1], Riccardo Moschetti[1], Bruno Murzi[1], Isabella Spadoni[1], Claudio Passino[1,3] and Aldo Clerico[1,3,]*

[1] Fondazione Toscana G. Monasterio, Massa, Italy
[2] CNR Institute of Clinical Physiology, Pisa, Italy
[3] Scuola Superiore Sant'Anna, Pisa, Italy

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 (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).

*Corresponding author: Prof. Aldo Clerico, MD, Laboratory of Cardiovascular Endocrinology and Cell Biology, Fondazione CNR – Regione Toscana G. Monasterio, Scuola Superiore Sant'Anna, Via Trieste 41, 56126 Pisa, Italy
E-mail: clerico@ifc.cnr.it
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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 preoperatively 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 bicaval 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, χ^2-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.

Characteristics, units	AKI (n=52)	Non AKI (n=83)	Total (n=135)
Age, months	3 (0–33)	11 (4–60)	7 (1–49)[a]
Male, %	30 (57.7)	48 (57.8)	78 (57.8)
Weight, kg	4.8 (3.4–12.3)	8.6 (4.9–17.0)	7 (3.8–13.4)[a]
Height, cm	57 (51.5–89.5)	72.5 (60.0–109.8)	67.5 (55.0–98.8)[a]
Body surface area, m^2	5.1 (4.8–6.1)	5.6 (5.2–6.7)	5.5 (5.0–6.4)[a]
Glenn, %	2 (3.85)	1 (1.20)	3 (2.24)
LRVO, %	–	4 (4.82)	4 (2.99)
Left ventricular pressure overload, %	8 (15.38)	9 (10.84)	17 (12.69)
Left ventricular volume overload, %	16 (30.77)	25 (30.12)	41 (30.6)
Palliated UH, %	2 (3.85)	4 (4.82)	6 (4.48)
Right ventricular pressure overload, %	12 (23.08)	12 (14.46)	24 (17.91)
Right ventricular volume overload, %	2 (3.85)	19 (22.89)	21 (15.67)
Transposition of the great arteries, %	5 (9.62)	3 (3.61)	8 (5.97)
Univentricular heart, %	5 (9.62)	6 (7.23)	11 (8.21)
BNP, ng/L	149 (54.5–938.0)	57 (35.0–116.8)	86 (40.8–216.8)[a]
Serum creatinine, mg/dL	0.24 (0.19–0.30)	0.24 (0.18–0.30)	0.24 (0.18–0.30)
Urinary creatinine, mg/dL	23.20 (7.55–54.88)	53.80 (20.55–91.70)	46.55 (16.09–78.20)[a]
u-NGAL, ng/mL	7.65 (3.55–27.18)	8.30 (3.70–20.95)	8.30 (3.7–21.5)
u-NGAL/urinary creatinine ratio	4.30 (1.60–9.35)	2.20 (0.63–6.18)	2.80 (0.75–7.03)
CPB time, min	153 (120–201.3)	98 (65–115)	112 (76–150)[a]
Cross-clamp, min	83 (29–122)	47 (27–67)	58 (28–83)[a]
Intubation time, h	48 (12–111)	8 (5–14)	12 (5–61)[a]
Inotropic time, h	108 (36–182)	12 (10–60)	36 (12–108)[a]
ICU stay, h	108 (36–156)	24 (12–36)	36 (12–108)[a]
Aristotle score	8.37 (2.00)	6.58 (2.18)	7.27 (2.28)[a]

[a]$p<0.05$ 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 outcome divided according to the different age-subgroups.

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

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 uNGALvalues 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 uNAGL 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

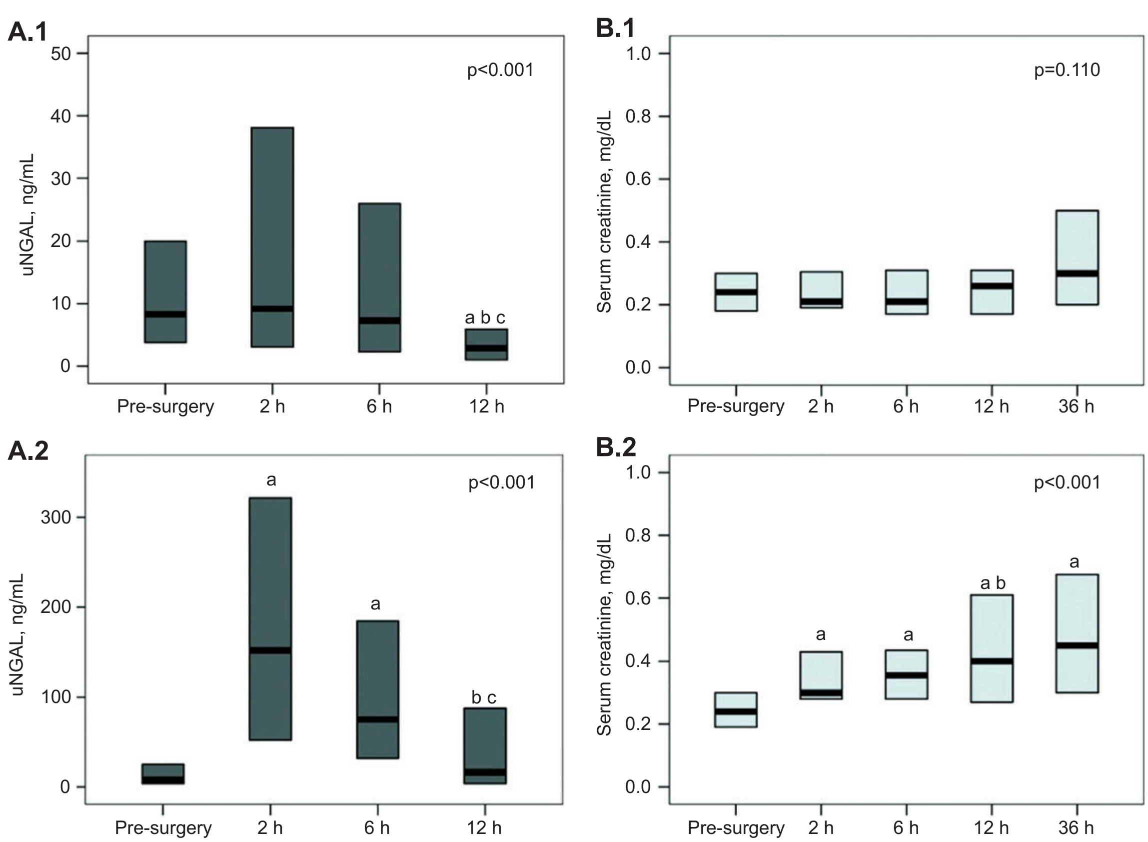

Figure 1 Behavior of uNGAL and serum creatinine values.
Behavior of uNGAL (Part A.1 and A.2) and serum creatinine (Part B.1 and B.2) levels in non-AKI group (upper panels) and AKI group (lower panels) of pediatric patients throughout the study. The results are reported as median (bold horizontal line) and interquartile range (box). The p-values for difference overall the samples collected at different times pre- and post-surgery (at 2, 6 and 12 h, respectively) were also reported. [a]p-value <0.05 for differences with pre-surgery levels; [b]p-value <0.05 for differences with 2 h levels; [c]p-value<0.05 for differences with 6 h levels.

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 (ρ=0.43, p<0.001 for uNGAL 2 h post-surgery) and time spent in the ICU (ρ=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),

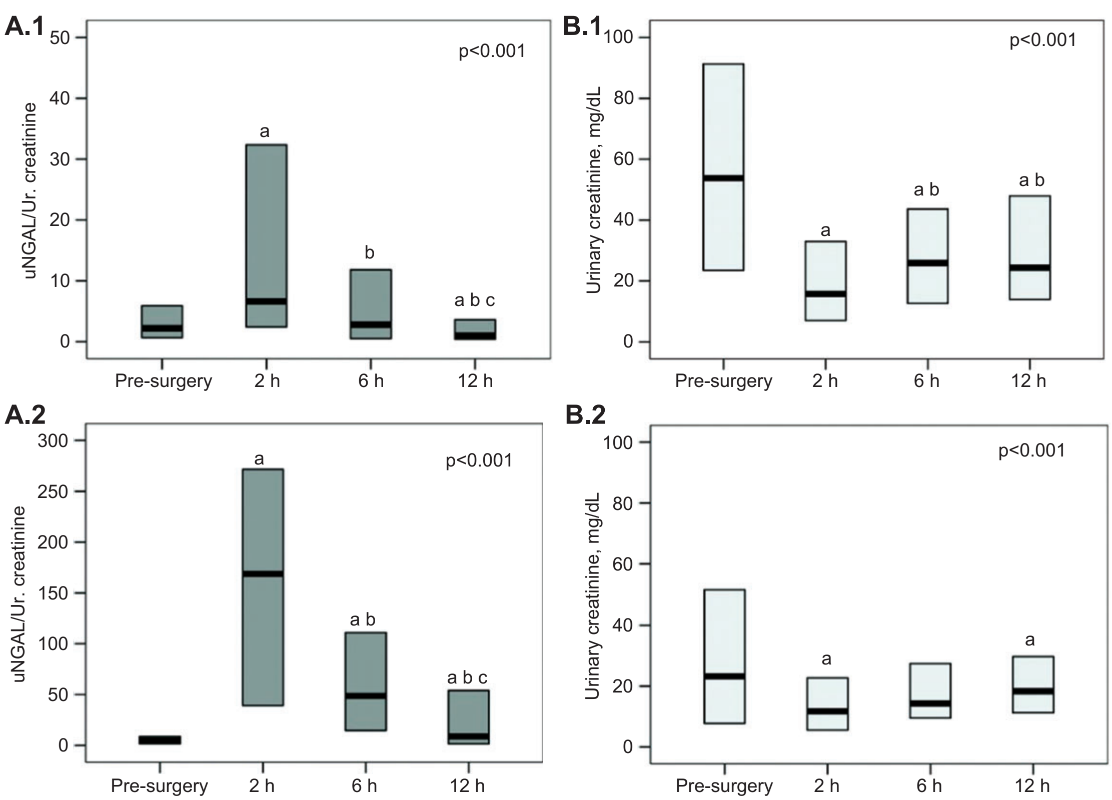

Figure 2 Behavior of uNGAl/urinary creatinine ratio and urinary creatinine values.
Behavior of uNGAl/urinary creatinine ratio (Part A.1 and A.2) and urinary creatinine (Part B.1 and B.2) levels in non-AKI group (upper panels) and AKI group (lower panels) of pediatric patients throughout the study. The results are reported as median (bold horizontal line) and interquartile range (box). The p-values for difference overall the samples collected at different times pre- and post-surgery (at 2, 6 and 12 h, respectively) were also reported. [a]p-value<0.05 for differences with pre-surgery levels; [b]p-value<0.05 for differences with 2 h levels; [c]p-value<0.05 for differences with 6 h levels. 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 3 Data of ROC analysis. The values of best cut-off value, AUC, sensitivity and specificity (calculated at the best cut-off) are reported.

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

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

length of stay in the ICU (ρ=0.42, p<0.001), and Aristotle score (ρ=0.24, p=0.007). BNP values at 12 h after surgery were also significantly correlated with 2 h uNGAL (ρ=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 (ρ=0.44, p<0.001), left ventricular ejection fraction (ρ=–0.30, p=0.007) and arterial blood pH (ρ=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).

Table 4 Logistic regression models for AKI.

Univariable models		
Variable, units	OR (CI 95%)	p-Value
Female vs. male	1.01 (0.50–2.03)	0.987
Body surface area, m^2	0.56 (0.36–0.86)	0.008
Older children vs. neonates	0.25 (0.10–0.62)	0.003
uNGAL pre-surgery, ng/mL[a]	1.07 (0.77–1.49)	0.677
uNGAL 2 h, ng/mL[a]	2.48 (1.81–3.40)	<0.001
uNGAL 6 h, ng/mL[a]	2.40 (1.74–3.30)	<0.001
uNGAL 12 h, ng/mL[a]	2.05 (1.53–2.75)	<0.001
uNGAL peak, ng/mL[a]	2.85 (1.99–4.08)	<0.001
uNGAL/urinary creatinine ratio pre-surgery[a]	1.29 (0.97–1.72)	0.081
uNGAL/urinary creatinine ratio 2 h[a]	1.87 (1.48–2.37)	<0.001
uNGAL/urinary creatinine ratio 6 h[a]	1.79 (1.42–2.25)	<0.001
uNGAL/urinary creatinine ratio 12 h[a]	1.85 (1.44–2.37)	<0.001
BNP pre-surgery, ng/L[a]	1.44 (1.14–1.82)	0.002
BNP 12 h, ng/L[a]	2.18 (1.43–3.34)	<0.001
CPB time, h	3.57 (2.01–6.33)	<0.001
Cross-clamp, h	3.66 (1.80–7.46)	<0.001
Intubation time, days	1.17 (1.04–1.31)	0.010
ICU stay, days	1.20 (1.07–1.35)	0.002
Aristotle score	1.55 (1.25–1.92)	<0.001
Multivariable model		
AUC (SE)=0.858 (0.040)		
uNGAL 2 h, ng/mL[a]	1.88 (1.30–2.72)	0.001
CPB time, h	1.42 (0.75–2.69)	0.281
Aristotle score	1.44 (1.05–1.99)	0.025

ICU, intensive care unit. [a]Log transformed variable. 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.

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.

As far as the diagnosis of AKI is concerned, a very recent study reported that plasma BNP is able to predict AKI in critically ill adult patients (28). In our clinical setting, only uNGAL values after surgery, together with CPB time and the Aristotle score, resulted in independent predictors of development of AKI syndrome, whereas BNP did not (Table 4). In particular, our results strongly support the

Table 5 Cox models for time to extubation (TTE).

Univariable models		
Variable, units	HR (CI 95%)	p-Value
Female vs. male	1.25 (0.87–1.81)	0.229
Body surface area, m^2	1.76 (1.46–2.12)	<0.001
Older children vs. neonates	2.49 (1.54–4.02)	<0.001
Urinary creatinine pre-surgery, mg/dL[a]	1.41 (1.12–1.79)	0.004
Urinary creatinine 2 h, mg/dL[a]	1.39 (1.13–1.71)	0.002
Urinary creatinine peak, mg/dL[a]	1.73 (1.31–2.28)	<0.001
Serum creatinine 12 h, mg/dL[a]	0.61 (0.45–0.84)	0.003
Serum creatinine peak, mg/dL[a]	0.58 (0.40–0.83)	0.003
uNGAL 2 h, ng/mL[a]	0.80 (0.72–0.89)	<0.001
uNGAL 6 h, ng/mL[a]	0.72 (0.63–0.81)	<0.001
uNGAL 12 h, ng/mL[a]	0.73 (0.64–0.83)	<0.001
uNGAL peak, ng/mL[a]	0.74 (0.66–0.83)	<0.001
uNGAL/creatinine ratio pre-surgery[a]	0.77 (0.66–0.9)	0.001
uNGAL/creatinine ratio 2 h[a]	0.76 (0.69–0.83)	<0.001
uNGAL/creatinine ratio 6 h[a]	0.70 (0.63–0.78)	<0.001
uNGAL/creatinine ratio 12 h[a]	0.71 (0.63–0.81)	<0.001
uNGAL/creatinine ratio peak[a]	0.74 (0.67–0.82)	<0.001
BNP pre-surgery, ng/L[a]	0.80 (0.70–0.91)	0.001
BNP 12 h, ng/L[a]	0.64 (0.52–0.78)	<0.001
CPB time, h	0.76 (0.64–0.90)	0.001
Cross-clamp, h	0.61 (0.46–0.82)	0.001
ICU stay, days	0.40 (0.32–0.51)	<0.001
Aristotle score	0.73 (0.67–0.81)	<0.001
Multivariable model		
Aristotle score	0.76 (0.68–0.84)	<0.001
BNP pre-surgery, ng/L[a]	0.82 (0.71–0.94)	0.004
uNGAL 2 h, ng/mL[a]	0.84 (0.75–0.94)	0.003

HR<1 suggested lower chance of extubation or conversely longer intubation time; HR<1 suggested higher chance of extubation or equally lower intubation time; ICU, intensive care unit. [a]Log transformed variable. 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.

hypothesis that uNGAL may allow an early diagnosis of AKI in pediatric patients with congenital heart defects within 6 h after cardiac surgery with a good diagnostic accuracy (Table 3). Taking into account that serum creatinine significantly increases only 36 h after surgery (Figure 2), our findings suggest that uNGAL can allow a correct diagnosis in about 80% of patients at least 1 day before than the serum creatinine. Conflicting results are reported in the literature about the diagnostic accuracy of NGAL as biomarker of AKI. Although the major part of studies (4–7, 9) suggested that NGAL, measured in urine or blood samples, is a useful biomarker of AKI, other authors (8) reported only a limited clinical value for plasma NGAL, assayed early after cardiac surgery in pediatric patients. However, Koch et al. (8) used plasma (rather than urine) samples and a point-of-care testing (POCT) method for the assay of NGAL. This POCT method shows a lower analytical sensitivity as compared to the immunoassay method used in this present study (18, 29). It is theoretically conceivable moreover, that the assay of circulating levels of NGAL may be less accurate than its measurement in urine (18), even if the results of a recent meta-analysis were not able to confirm this hypothesis (19). Finally, the independent and significant contribution of CPB time on the prediction of the presence of AKI (Table 4) is largely expected when considering that CPB causes complex systemic inflammatory responses that significantly contribute to several adverse postoperative complications, including renal damage (30, 31).

Study strengths and limitations of the present study

This study has several strengths. Firstly, we used a prospective cohort design and a rigorous protocol to collect specimens. Our study protocol furthermore, which used several sampling times, was able to clearly define the time course of the biomarkers. Finally, for the first time, we evaluated together an early marker of renal damage (i.e., NGAL) and a marker of cardiac function (i.e., BNP) in order to test the hypothesis that the combined use of these two biomarkers could provide a better risk stratification in pediatric patients with congenital heart defects undergoing cardiac surgery.

This study, however, does also have some limitations. Firstly, the total number of studied patients is relatively low. It is important however, to note that congenital heart defects, especially those with a more complex severe disease, are very rare (only about 1: 10,000 neonates present this disease) (32). It is consequently, very difficult to enrol a very large number of patients with congenital heart defects in a short time span. Another limitation is that our study considered outcome measures limited to the peri-operative period. Further studies with longer follow-up are needed to evaluate the long-term prognostic value of uNGAL.

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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