Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery

Jaya Mishra*, Catherine Dent*, Ridwan Tarabishi*, Mark M Mitsnefes, Qing Ma, Caitlin Kelly, Stacey M Ruff, Kamyar Zahedi, Mingyuan Shao, Judy Bean, Kiyoshi Mori, Jonathan Barasch, Prasad Devarajan

Summary

Background The scarcity of early biomarkers for acute renal failure has hindered our ability to launch preventive and therapeutic measures for this disorder in a timely manner. We tested the hypothesis that neutrophil gelatinase-associated lipocalin (NGAL) is an early biomarker for ischaemic renal injury after cardiopulmonary bypass.

Methods We studied 71 children undergoing cardiopulmonary bypass. Serial urine and blood samples were analysed by western blots and ELISA for NGAL expression. The primary outcome measure was acute renal injury, defined as a 50% increase in serum creatinine from baseline.

Findings 20 children (28%) developed acute renal injury, but diagnosis with serum creatinine was only possible 1–3 days after cardiopulmonary bypass. By contrast, urine concentrations of NGAL rose from a mean of 1·6 μg/L (SE 0·3) at baseline to 147 μg/L (23) 2 h after cardiopulmonary bypass, and the amount in serum increased from a mean of 3·2 μg/L (SE 0·5) at baseline to 61 μg/L (10) 2 h after the procedure. Univariate analysis showed a significant correlation between acute renal injury and the following: urine and serum concentrations of NGAL at 2 h, and cardiopulmonary bypass time. By multivariate analysis, the amount of NGAL in urine at 2 h after cardiopulmonary bypass was the most powerful independent predictor of acute renal injury. For concentration in urine of NGAL at 2 h, the area under the receiver-operating characteristic curve was 0·998, sensitivity was 1·00, and specificity was 0·98 for a cutoff value of 50 μg/L.

Interpretation Concentrations in urine and serum of NGAL represent sensitive, specific, and highly predictive early biomarkers for acute renal injury after cardiac surgery.

Introduction Acute renal failure represents a very important and potentially devastating disorder in clinical medicine. Its prevalence varies from 5% of all patients admitted to hospital to 30–50% of those in intensive-care units. Despite substantial technical improvements in treatments, mortality and morbidity associated with acute renal failure remain dismally high.

Renal ischaemia-reperfusion injury is the leading cause of acute renal failure in the native and transplanted kidney. Advances in basic science research have highlighted the pathogenesis of such injury and have paved the way for successful therapeutic approaches in animal models. However, translational research efforts in patients have yielded disappointing results. A major reason for the failure to find an effective treatment in patients is the scarcity of early biomarkers for acute renal failure, akin to troponins in acute myocardial disease, and hence an unacceptable delay in initiating any treatment regimens. Indeed, work done in patients has established that the earlier the intervention, the better the chance of ameliorating the renal dysfunction.

In current clinical practice, acute renal failure is typically diagnosed by measuring serum creatinine. Unfortunately, creatinine is an unreliable indicator during acute changes in kidney function. First, serum creatinine concentrations might not change until about 50% of kidney function has already been lost. Second, serum creatinine does not accurately depict kidney function until a steady state has been reached, which could take several days. However, work in animals has shown that although acute renal failure due to ischaemia can be prevented, treated, or both by several techniques, these must be started very early after the renal injury.

Acute renal dysfunction occurs in up to 40% of adults after cardiac surgery, with 1–5% needing dialysis, in whom the mortality rate approaches 80%. Pathophysiological mechanisms include diminished renal blood flow, loss of pulsatile flow, hypothermia, atheroembolism, and a generalised inflammatory response. Various clinical algorithms have been proposed for prediction of acute renal failure needing dialysis, based on preoperative risk factors, but no methods are available for the early diagnosis of lesser degrees of renal injury. Acute renal failure also complicates up to 10% of cardiac surgical procedures in infants and children with congenital heart disease. This population is especially vulnerable to development of acute renal failure since many children need multiple surgical procedures for step-by-step repair of complex congenital anomalies. However, these children are unique in that comorbid conditions such as advanced age, atherosclerotic disease, and diabetes are usually absent, making them an ideal group for investigation of biomarkers as predictors of early ischaemic renal injury.
We have previously used a genome-wide interrogation strategy to identify kidney genes that are induced very early after ischaemia in animal models, whose protein products might serve as novel biomarkers for the initiation phase of acute renal failure.17 We identified neutrophil gelatinase-associated lipocalin (NGAL) as one of the most strikingly upregulated genes (HUGO-approved gene name \( LCN2 \)) and overexpressed proteins in the kidney after ischaemia.17-19 NGAL was easily detected in urine early after ischaemia in mouse and rat models.18 We therefore tested the hypothesis that NGAL represents an early biomarker of ischaemic renal injury in children undergoing cardiac surgery.

**Patients and methods**

**Patients**

This investigation was approved by the institutional review board of the Cincinnati Children’s Hospital Medical Center. All children undergoing cardiopulmonary bypass for surgical correction of congenital heart disease between January, 2004, and November, 2004, were prospectively enrolled. We obtained written informed consent from the legal guardian of every child before enrolment. Exclusion criteria included pre-existing renal insufficiency, diabetes mellitus, peripheral vascular disease, and use of nephrotoxic drugs before or during the study period. We therefore studied a homogeneous population of children with very possibly no major confounding variables, in whom the only obvious renal insult would be the result of ischaemia-reperfusion injury after cardiopulmonary bypass.

**Procedures**

To minimise postoperative volume depletion, all children received at least 80% of their maintenance fluid requirements during the first 24 h after surgery and 100% maintenance subsequently. We took spot urine and blood samples at baseline and at frequent intervals for 5 days after cardiopulmonary bypass. Urine samples were obtained every 2 h for the first 12 h and then once every 12 h. We collected blood samples at 2 h after cardiopulmonary bypass, every 12 h for the first day, and then once daily for 5 days. When the cardiopulmonary bypass time exceeded 2 h, the first postoperative urine and serum samples were obtained at the end of cardiopulmonary bypass, and this sample was regarded as the 2-h sample. We also took urine and blood samples from healthy adult volunteers for establishment of normal NGAL values. We centrifuged samples at 2000 \( \times \) g for 5 min and stored the supernatants in equal volumes at –80ºC. Serum creatinine was measured at baseline and routinely monitored at least twice a day in the immediate postoperative period, and at least daily after postoperative day 3.

The primary outcome variable was development of acute renal injury, defined as a 50% or greater increase in serum creatinine from baseline. Other variables we obtained included age, sex, ethnic origin, cardiopulmonary bypass time, previous heart surgery, urine output, and urine creatinine.

**Expression of recombinant human NGAL**

Recombinant human NGAL was produced in a bacterial expression system for use as standards in western blot and ELISA procedures. Briefly, the coding region of \( LCN2 \), minus its leader sequence and stop codon, was amplified by PCR, using a full-length human \( LCN2 \) cDNA clone purchased from American Type Culture Collection (Manassas, VA, USA). The oligonucleotide primers used for amplification (panel) were based on those previously published.20 The PCR product was sequenced to confirm its identity, cloned into the pGEX-4T-3 expression vector system (Pharmacia, Piscataway, NJ, USA), expressed as a fusion protein with glutathione S-transferase in bacteria, affinity purified by adsorption to glutathione sepharose 4B beads (Pharmacia), and released by cleaving the adsorbed fusion protein with human thrombin (2·5 U/mL, Sigma, St Louis, MO, USA).20 The purified protein was assessed by size fractionation.

**Western-blot analysis for NGAL expression and quantitation**

Equal volumes (30 \( \mu \)L) of every urine sample were boiled for 10 min in denaturing buffer and subjected to standard western-blot analysis with an affinity-purified goat polyclonal antibody raised against human NGAL (F-19, Santa Cruz Biotechnology, Santa Cruz, CA, USA). We prepared simultaneous blots under identical conditions of transfer and exposure with known quantities of recombinant human NGAL, as standards for quantitation of urine NGAL.21 The laboratory investigators were unaware of the sample sources and clinical outcomes until the end of the study.

**ELISA for NGAL quantitation**

Clinical use of immunoblot-based techniques for rapid detection of biomarkers for acute renal injury is limited by time and variations in assay conditions. We envisioned that the establishment and validation of a sandwich monoclonal ELISA procedure for NGAL in human urine and serum would represent a substantial advance. We modified previously published protocols for detection of NGAL derived from neutrophils.22 Briefly, microtitre plates were coated overnight at 4ºC with a mouse monoclonal antibody raised against human NGAL (HYB211-05, AntibodyShop, Gentofte, Denmark). We obtained included age, sex, ethnic origin, cardiopulmonary bypass time, previous heart surgery, urine output, and urine creatinine.

**Panel: Primers used in the study**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>NGAL1:</td>
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</tr>
<tr>
<td>NGAL2:</td>
<td>5’CGGAATTCTCAAGCGTCGATACCTGATCCC3’</td>
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</table>
Denmark). All subsequent steps were undertaken at room temperature. Plates were blocked with buffer containing 1% bovine serum albumin, coated with 100 μL of samples (urine or serum) or standards (NGAL concentrations ranging from 1–1000 μg/L), and incubated with a biotinylated monoclonal antibody against human NGAL (HYB211-01B, AntibodyShop) followed by avidin-conjugated horseradish peroxidase (Dako, Carpinteria, CA, USA). Tetramethylbenzidine substrate (BD Biosciences, San Jose, CA, USA) was added for colour development, which was read after 30 min at 450 nm with a microplate reader (Benchmark Plus, BioRad, Hercules, CA, USA). All measurements were made in triplicate and in a blinded fashion.

**Statistical analysis**
SAS version 8.2 was used for analyses. To compare continuous variables, we used a two-sample t test or Mann-Whitney rank sum test, and to compare categorical variables we used the $\chi^2$ or Fisher’s exact test, as indicated. To measure the sensitivity and specificity for urine and serum NGAL at different cutoff values, a conventional receiver-operating characteristic (ROC) curve was generated for urine NGAL at 2 h and 4 h after cardiopulmonary bypass and for serum NGAL at 2 h after the procedure. We calculated the area under the curve to ascertain the quality of NGAL as a biomarker. An area of 0.5 is no better than expected by chance, whereas a value of 1.0 signifies a perfect biomarker. Univariate and multivariate stepwise multiple logistic regression analyses were undertaken to assess predictors of acute renal injury. Potential independent predictor variables included age, sex, ethnic origin, cardiopulmonary bypass time, previous heart surgery, urine output, urine NGAL at 2 h after cardiopulmonary bypass, and serum NGAL at 2 h after cardiopulmonary bypass. We judged $p<0.05$ significant.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Without acute renal injury (n=51)</th>
<th>Acute renal injury (n=20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>4.0 (0.7)</td>
<td>2.1 (1.2)</td>
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<tr>
<td>Boys</td>
<td>32</td>
<td>13</td>
<td>0.792</td>
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<tr>
<td>White ethnic origin</td>
<td>45</td>
<td>17</td>
<td>0.705</td>
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**Clinical outcomes**

<table>
<thead>
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<th></th>
<th>Without acute renal failure (n=51)</th>
<th>With acute renal failure (n=20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous heart surgery</td>
<td>15</td>
<td>5</td>
<td>0.778</td>
</tr>
<tr>
<td>Cardiopulmonary bypass time (min)</td>
<td>105 (8.6)</td>
<td>179 (13.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Change in serum creatinine (%)</td>
<td>7.7 (1.8)</td>
<td>99 (9.3)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Diagnosis**

- Ventricular septal defect
- Tetralogy of Fallot
- Atrial septal defect
- Coarctation of aorta
- Aortic stenosis
- Hypoplastic left heart
- Atrioventricular canal
- Pulmonic stenosis
- Transposition of the great arteries
- Truncus arteriosus
- Double-outlet right ventricle
- Anomalous left coronary artery
- Cor triatriatum
- Left-ventricular outflow tract obstruction
- Mitral regurgitation
- Aortic regurgitation

Data are mean (SE) or number of children.

**Table 1: Patients’ characteristics and clinical outcomes**

**Figure 1: Analysis of urine NGAL by western blot**
(A) Representative western blot of urine samples obtained at various timepoints after cardiopulmonary bypass from a patient who subsequently developed acute renal failure. Both blots were probed with a monoclonal antibody to human NGAL. (B) Mean urine NGAL concentrations at various timepoints after cardiopulmonary bypass (upper) and corrected for urine creatinine excretion (lower). Error bars are SE.
Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

100 children were considered for participation in the study. 29 were excluded because of nephrotoxin use (ibuprofen, angiotensin-converting-enzyme inhibitors, gentamicin, vancomycin) before or soon after surgery. Thus, 71 children were included in the study.

Acute renal injury occurred in 20 children (28%) within a 3-day period. Of these, serum creatinine rose 24–48 h after cardiopulmonary bypass in eight, but in the other 12 the increase happened 48–72 h after the procedure. Thus, the diagnosis of acute renal injury using currently accepted practices could be made only days after the inciting event.

Based on the primary outcome, we classified children into those with and without acute renal injury. No differences were noted between the two groups with respect to sex, ethnic origin, or urine output (table 1). Children who developed acute renal injury were younger and had longer cardiopulmonary bypass times compared with those who did not develop acute renal injury. Acute renal injury was more frequent in children with an underlying diagnosis of hypoplastic left heart and tetralogy of Fallot, and was less frequent or absent in those with atrial septal defect, ventricular septal defect, or valvular heart disease.

Western-blot analysis of urine samples from children with acute renal failure at various timepoints after cardiopulmonary bypass showed one band of expected size (28 kDa), which was specifically recognised by a monoclonal antibody to human NGAL (figure 1). NGAL was virtually undetectable in the urine of all children before surgery and in ten healthy adult volunteers. In the 51 children who never developed acute renal injury, a small increase was noted in urinary NGAL at 2 h after cardiopulmonary bypass or with the first available sample (p=0.005 vs baseline) and 4 h after surgery (p=0.005 vs baseline). By contrast, those who subsequently developed acute renal injury had a striking rise in urinary NGAL at all timepoints (p<0.0001 vs baseline; figure 1). The pattern of urinary NGAL excretion was characterised by a peak very early after the precipitating event followed by a lesser but sustained increase over the entire duration of the study. This overall pattern remained unchanged when urinary NGAL concentration was normalised for urinary creatinine excretion.

We developed a sensitive and reproducible ELISA for NGAL, to provide accurate quantitation of samples and to confirm data obtained by western-blot analysis. The ELISA results very closely paralleled those obtained by
western-blot analysis, with a difference of less than 20%. Urine NGAL concentrations were consistently low in ten healthy volunteers (mean 2·2 μg/L [SE 0·5]) and at baseline in all children (1·6 μg/L [0·3]). In the 51 who never developed acute renal injury, a small increase in urinary NGAL was noted at 2 h (p=0·003 vs baseline) and 4 h (p=0·002 vs baseline) after cardiopulmonary bypass. Children who subsequently developed acute renal injury displayed a remarkable increase in urinary NGAL at all timepoints (p<0·0001 vs baseline; figure 2). Urinary NGAL excretion peaked very early after cardiopulmonary bypass, followed by a lesser but sustained increase over the entire duration of the study. This overall pattern remained consistent when urinary NGAL concentration was normalised for urinary creatinine excretion (figure 2). A scatterplot of the first available postoperative urine NGAL measurements showed that all children who subsequently developed acute renal injury had a concentration of urinary NGAL above an arbitrary cutoff value of 50 μg/L, whereas only one of 51 controls had a value above this arbitrary cutoff.

Serum NGAL concentrations were consistently low in six healthy adult volunteers (mean 2·5 μg/L [SE 0·8]) and all children before surgery (3·2 μg/L [0·5]). Children who never developed acute renal injury had a small increase in serum NGAL at 2 h or with the first available sample after cardiopulmonary bypass (p=0·001 vs baseline; figure 3). Those who subsequently developed acute renal injury had a striking increase in serum NGAL at all timepoints (p<0·0001 vs baseline). Similar to urine concentrations, the amount of NGAL in serum peaked very early after surgery, followed by a lesser but sustained increase over the entire duration of the study. A scatterplot of all the earliest serum NGAL measurements (2 h after surgery) showed that none of the 51 children who never developed acute renal injury had a value above an arbitrary cutoff of 50 μg/L, whereas ten of 20 children who developed acute renal injury had a concentration in serum above this value.

Univariate analysis of our data showed that the following outcomes were not predictive of acute renal injury: age, sex, ethnic origin, previous surgery, and urine output. A significant correlation was found between acute renal injury (50% or greater in serum creatinine) and the following: urine NGAL at 2 h or the first available sample after cardiopulmonary bypass (r=0·79, p=0·002), serum NGAL at 2 h or the first available sample after surgery (r=0·64, p=0·03), and duration of cardiopulmonary bypass (r=0·49, p=0·02). However, by multiple stepwise regression analysis, only urine NGAL at 2 h after surgery emerged as the most powerful independent predictor of acute renal injury in this cohort (r=0·76, p<0·0001).

For urine NGAL, the area under the ROC curve was 0·998 at 2 h after cardiopulmonary bypass (figure 4). Table 2 lists the derived sensitivities, specificities, and predictive values at different cutoff concentrations. For urine NGAL, a cutoff of either 25 or 50 μg/L yielded good sensitivity and specificity at 2 h and 4 h after surgery. For serum NGAL at 2 h after cardiopulmonary bypass, sensitivity and specificity were best at the 25 μg/L cutoff.

**Discussion**

We have shown that the concentration of NGAL in urine and serum is strikingly raised in children with acute renal failure after cardiopulmonary bypass. Human NGAL is a 25 kDa protein covalently bound to gelatinase from human neutrophils. It is generally expressed at very low concentrations in several human tissues, including kidney, trachea, lungs, stomach, and colon. NGAL expression is induced in injured epithelia; for example, concentrations are raised in the serum of
patients with acute bacterial infections, in the sputum of those with asthma or chronic obstructive pulmonary disease, and in bronchial fluid from the emphysematous lung.25

In the post-ischaemic kidney, NGAL is upregulated in several nephron segments, and the protein accumulates predominantly in proximal tubules, where it colocalises with proliferating epithelial cells.18 These findings suggest that NGAL might be expressed by the damaged tubule to induce re-epithelialisation. In support of this hypothesis is the identification of NGAL as a regulator of epithelial morphogenesis in cultured kidney tubule cells26 and as an iron-transporting protein during nephrogenesis.27 Delivery of iron into cells is crucial for cell growth and development and is presumably also important for renal regeneration after ischaemic injury. Indeed, findings indicate that exogenously administered NGAL ameliorates ischaemic acute renal injury in mice by tilting the balance of tubule cell fate towards survival.28 Thus, NGAL has emerged centre-stage in the area of acute renal failure research, not only as a novel biomarker but also as an innovative therapeutic method.

Our study has several strengths. First, we prospectively recruited a homogeneous cohort of children in whom renal ischaemia-reperfusion injury arose during surgical correction of congenital cardiac disease. These children did not have common comorbid variables such as atherosclerotic disease, diabetes, and nephrotoxin use, all of which can confound and hinder the identification of early biomarkers for ischaemic acute renal injury. All children started with normal kidney function and essentially undetectable concentrations of NGAL in the urine and serum, just like healthy adult volunteers. This study design allowed us to determine the precise timing of NGAL appearance in the urine and serum after cardiopulmonary bypass. Our results indicate that NGAL is not only a powerful immediate early biomarker for acute renal injury, preceding any increase in serum creatinine by 1–3 days, but is also a valid discriminatory marker for the entire duration of the study.

Second, we established and validated an ELISA procedure for NGAL in human urine and serum with commercially available antibodies, to confirm immunoblot findings and accurately quantify changes in NGAL. Standard curves were straight in the 1–1000 μg/L range, which covers the entire spectrum of NGAL concentrations detected in our study. Interassay and intra-assay coefficient variations for NGAL were less than 5%, indicating good reliability. The ELISA results also provide a rationale and framework for the commercial design of point-of-care diagnostic kits for NGAL, pending further analysis of preanalytical and analytical factors that might impair the performance of this method.

Finally, we simultaneously studied both urine and serum samples. Although urinary diagnostics have several advantages, including the non-invasive nature of sample collection and few interfering proteins, some disadvantages also exist. These include difficulty in obtaining samples from patients with severe oliguria, potential changes in urinary biomarker concentration induced by the overall fluid status and diuretic therapy, and the fact that several urinary biomarkers have in the past shown insufficient sensitivity or specificity.1 Serum-based diagnostics have revolutionised intensive-care medicine. Examples include measurement of troponins for early diagnosis of and timely interventions in acute myocardial infarction29 and the prognostic value of

<table>
<thead>
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<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
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<tr>
<td>Cutoffs for 2-h urine NGAL</td>
<td></td>
<td></td>
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<tr>
<td>25 μg/L</td>
<td>1.00</td>
<td>0.98</td>
<td>0.95</td>
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<tr>
<td>50 μg/L</td>
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<td>0.95</td>
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<td>80 μg/L</td>
<td>0.90</td>
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<td>1.00</td>
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<td>100 μg/L</td>
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<td>25 μg/L</td>
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<td>80 μg/L</td>
<td>0.20</td>
<td>1.00</td>
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Table 2: NGAL test characteristics at different cutoff values

Figure 4: ROC curve analysis
B-type natriuretic peptide in patients with acute coronary syndrome. As far as we know, NGAL is the only biomarker that has been investigated in both serum and urine for the early diagnosis of ischaemic renal injury.

Our results compare favourably with those obtained for several other biomarkers of ischaemic renal injury. Most studies reported thus far have been retrospective, have examined biomarkers in the established phase of acute renal failure, and have been restricted to the urine and to only one method of detection. Several tubular proteins have been measured in the urine, with conflicting and unsatisfactory results. Kidney injury molecule 1, a kidney-specific adhesion molecule, is detectable by ELISA in the urine of patients with established acute tubular necrosis. Also, the sodium-hydrogen exchanger isoform 3 has been shown by western-blot analysis to be increased in the membrane fractions of urine from patients with established acute renal failure. However, the sensitivity and specificity of these biomarkers for the detection of renal injury have not been reported. Of the inflammatory cytokines involved in acute renal failure, raised concentrations of urinary interleukin 6, interleukin 8, and interleukin 18 have been recorded in patients with delayed graft function after cadaveric kidney transplants.

As far as we are aware, no biomarkers have been investigated prospectively for appearance in urine during the pathogenesis of ischaemic acute renal failure. Findings of a prospective study showed that a rise in serum cystatin C precedes the increase in serum creatinine in a select population at high-risk for development of acute renal failure. However, the disorder in these subjects was multifactorial, due to a combination of ischaemic, prerenal, nephrotoxic, and septic causes. Furthermore, since cystatin C is a marker of glomerular filtration rate, serum concentrations might rise only after the rate begins to fall. On the other hand, NGAL is rapidly induced in kidney tubule cells in response to ischaemic injury, and its early appearance in the urine and serum is independent of the glomerular filtration rate, but is highly predictive of a fall in glomerular filtration rate that might happen several days later.

Our results showing a small transient increase in urine and serum NGAL in children who did not develop acute renal failure accord with previous observations that cardiopulmonary bypass surgery leads to release of NGAL into the circulation, probably secondary to inflammatory activation of leukocytes initiated by the extracorporeal circuit.

A limitation of our study is that it is a single-centre analysis of children with congenital heart disease and predominantly ischaemic kidney injury. Although this cohort was intentionally chosen to eliminate common confounding variables and comorbid conditions, we acknowledge that acute renal failure is frequently multifactorial, and our results will need to be validated in a larger population in whom additional mechanisms of renal injury might be invoked.

Simultaneous investigation of several urinary and serum proteins as early biomarkers and predictors of acute renal failure will also be important. Not one biomarker but a collection of strategically selected proteins might provide the hitherto elusive panel for early and rapid diagnosis of acute renal injury. The present study identifies NGAL as a prime candidate for inclusion in such a panel. Such a method would be indispensable for the timely institution of potentially effective treatments in early human acute renal failure, a common clinical disorder still associated with a dismal prognosis for which intervention is desperately needed.

Contributors
P Devarajan, in collaboration with C Dent and J Barasch, had the idea for and designed the study. J Mishra, R Tarabishi, Q Ma, C Kelly, S M Ruff, K Mori, and K Zahedi did the experiments. M Mitsnefes, J Bean, and M Shao did the statistical analysis. All authors contributed to data interpretation and wrote the report.

Conflict of interest statement
We declare that we have no conflict of interest.

References