Neutrophil gelatinase-associated lipocalin as a marker of postoperative acute kidney injury following cardiac surgery in patients with pre-operative kidney impairment

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Abstract

Introduction: Acute kidney injury (AKI) is a serious complication of cardiac surgery. The current 'gold standard' for determining AKI is change in serum creatinine and urine output, however, this change occurs relatively late after actual injury occurs. Identification of new biomarkers that detect early AKI is required. Recently new biomarkers, such as the NephroCheck® Test and AKIRisk have also been tested and found to be good indicators of AKI. Neutrophil gelatinase-associated lipocalin (NGAL) has shown promise in paediatric patients but has displayed varied results in adult populations, particularly post cardiac surgery. The aim of this study was to assess the value of urinary NGAL as a biomarker of AKI in patients with pre-existing renal impairment (eGFR >15ml/min to eGFR<60ml/min).

Methods: A post-hoc analysis of urinary NGAL concentrations from 125 patients with pre-existing kidney impairment, who participated in a randomised trial of haemofiltration during cardiac surgery, was undertaken. Urinary NGAL was measured using ELISA at baseline, post-operatively and 24 and 48 hours after surgery and serum creatinine was measured pre and post operatively and then at 24, 48, 72 and 96 hours as routine patient care. NGAL concentrations were compared in patients with and without AKI determined by changes in serum creatinine concentrations. A Kaplan-Meier plot compared survival for patients with or without AKI and a Cox proportional hazards analysis was performed to identify factors with the greatest influence on survival.

Results: Following surgery 43% of patients developed AKI (based on KDIGO definition). Baseline urinary NGAL was not found to be significantly different between patients that did and did not develop AKI. Urinary NGAL concentration was increased in all patients following surgery, regardless of whether they developed AKI and was also significant between groups at 24 ($p=0.003$) and 48 hours ($p<0.0001$). Urinary NGAL concentrations at 48 hours correlated with serum creatinine

concentrations at 48 hours (r=0.477, p<0.0001), 72 hours (r=0.488, p<0.0001) and 96 hours (r=0.463, p<0.0001). Urinary NGAL at 48 hours after surgery strongly predicted AKI (AUC=0.76; P=0.0001). A Kaplan-Meier plot showed that patients with postoperative AKI had a significantly lower 7-year survival compared with those without AKI. Postoperative urinary NGAL at 48 hours >156ng/mL also strongly predicted 7 year survival. However, additive EuroSCORE, age, current smoking and post-operative antibiotics usage were distinctly significantly more predictive of 7-year survival as compared with postoperative urinary NGAL at 48 hours >156ng/mL.

Conclusions: Our study demonstrated that postoperative urinary NGAL levels at 48 hours post-surgery strongly predicts the onset or severity of postoperative AKI based on KDIGO classification in patients with preoperative kidney impairment and was also strongly related to 7-year survival.

Keywords

Acute kidney injury; NGAL; cardiac surgery; renal impairment

Introduction

Acute kidney injury (AKI) is a serious complication of cardiac surgery, and is one of the most common causes of AKI in the intensive care unit [1]. Up to 39% of patients have been reported to develop AKI depending on patient characteristics and the type of surgery they underwent [[2]. Patients that go on to develop AKI following cardiac surgery have an increased hospital stay, increased risk of chronic kidney disease and an increased risk of death within 5 years post-surgery [3].

The development of postoperative AKI is known to be driven by a number of intraoperative factors such as decreased renal perfusion, embolic events, cardiopulmonary bypass-induced inflammation, nephrotoxins and reperfusion injury [4-6] [16-18]. Studies have shown that only about 1% of cardiac surgery patients that develop severe postoperative AKI receive dialysis peri-operatively [7] [19]. It has been estimated that over 15% of patients develop postoperative AKI [8-11] [20-23]. Although

these patients are not treated with dialysis they are still at a higher risk of short- and long-term mortality, a longer hospital stay, and higher health care costs [8-11] [20-23]. The current 'gold standard' for kidney injury is based on changes in serum creatinine, however, this is a marker of kidney function and changes are only detected relatively late after actual injury occurs. As such, new markers that can identify post-operative AKI earlier are required.

Changes in serum creatinine concentrations are currently the 'gold standard' in assessing kidney injury. However, changes in serum creatinine occur as a result of the functional change in glomerular filtration rate, due to structural changes that occur during the early stage of kidney injury. As such, these changes can only be detected relatively late after the initial injury occurs. It can also be influenced by several factors in the absence of kidney injury including age, gender, muscle mass, muscle metabolism, medications, hydration status, nutrition status and tubular secretion. As a result there is a need for clinical biomarkers that can detect AKI earlier, and more accurately, so that supportive therapy can be initiated in a time effective manner to the right patients. Many of these new biomarkers have concentrated on biomarkers of tubular injury such as neutrophil gelatinase-associate lipocalin (NGAL).

NGAL is a small, 25kDa, protein that is rapidly up-regulated and excreted following acute tubular damage [12-13] and can be detected in both plasma and urine early in the process of kidney injury. Studies in paediatric patients have indicated that a rise in NGAL can be detected as soon as 2-6 hours following surgery in both serum and urine [14-15] and as such it would be a useful biomarker in evaluating and treating patients earlier, potentially leading to better outcomes. However, research in different adult populations has shown conflicting results [16-19] , indicating that changes in serum biomarker concentrations are not necessarily related to decreased kidney function alone and can be the product of a systemic response [20]. In contrast, urine samples can be noninvasively collected, contain a reduced number of interfering proteins, and may have higher specificity for kidney damage irrespective of systemic pathology [21]. The aim of this study was therefore, to

assess the value of NGAL as a biomarker of AKI in patients with pre-existing renal impairment (eGFR <60ml/min) following cardiac surgery.

Methods

In total 199 patients were recruited into a randomised trial of haemofiltration on bypass [20]. In this post-hoc analysis, 74 patients were excluded due to incomplete sample sets. All participants gave written informed consent. Men and women at least 18 years old were included if they were high-risk patients undergoing elective surgery for on-pump surgery, i.e. valve replacement, coronary artery bypass graft (CABG) or combined CABG and valve procedures. They had an impaired renal function established pre-operatively by an eGFR <60ml/min. Patients were recruited from the routine waiting list for cardiac operations and were pre-screened for inclusion/exclusion criteria. Patients were excluded if they were scheduled to undergo surgery with anticipated CPB time less than 60 minutes, undergoing surgery on the great vessels (aortic surgery, had impaired liver function (serum bilirubin >60 or INR >2 without anticoagulation), patients further down the line of renal failure (eGFR <15ml/min) or were on dialysis, had malignancy or those that were pregnant. Informed patient consent was obtained from patients attending their routine pre-operative appointments, according to the protocol approved by the National Research Ethics Service, Northwest 4 Research Ethics Committee UK, and conducted in accordance with the Declaration of Helsinki and the European Union Clinical Trials Directive 2001/20/EC. Details of the surgical and anaesthetic protocols are as described previously [22].

Anaesthesia

Anaesthetic management was as per individual consultant's preference. All anaesthetics were opioid-based with anaesthesia being induced with either a benzodiazepine or propofol (AstraZeneca, London, UK). Muscle relaxation was maintained with Vecuronium (Merck, Sharp & Dohme, Ltd,

Hertfordshire, UK) and anaesthesia was maintained using isoflurane (Baxter UK, Newbury, Berkshire, UK) in oxygen/air. Depth of anaesthesia was monitored continuously in all patients using bispectral index-monitoring. Inotrope requirements were at the discretion of the individual consultants, but all inotropes used were recorded within the case record

Surgery

In all cases, cardiopulmonary (CPB) bypass surgery was performed through a median sternotomy. Myocardial protection was based on surgical preferences, with a choice between intermittent cold blood and intermittent cold crystalloid cardioplegia. The delivery route was antegrade only or antegrade followed by retrograde. Some surgeons preferred to complete all anastomoses proximal and distal while the aortic cross clamp was still on. Others preferred to do the proximal anastomoses with just a side clamp on (once the aortic cross clamp has been removed) and some used a variation of the above depending on the number of grafts and condition of the aorta. A standard 1.3 m^2 haemofiltration set was used for Z-BUF kit that was supplied by Chalice Medical, UK (Worksop, Nottinghamshire) and connected to the pump.

Critical care unit

Patients were admitted and cared for in the immediate perioperatively period in the Intensive care unit (ICU). Indications for postoperative haemofiltration were: hyperkalaemia (potassium >6.0 mmol/L); metabolic acidosis of renal origin; and anuria or oliguria urine output of less than 20 ml/hour for more than 6 hours (despite adequate filling and adequate cardiac output) resulting in clinically significant fluid overload. The choice of use of inotropes and their duration was left to the discretion of ICU clinicians who also made the clinical decisions regarding appropriate time to discharge patients from the ICU. In-patients' follow-up monitoring started on the day of surgery until hospital discharge or up to the 30th day of consecutive hospital stay before discharge.

Sample collection and measurements

At least 10ml of urine was collected for biomarker analysis from patients at 4 time points: prior to surgery, within 6 hours on ICU and at 24 and 48 hours post-operatively. The samples were centrifuged at 1000xg for 10 minutes, aliquoted and frozen at -80°C for further analysis. Urinary NGAL concentrations were then measured using commercial enzyme-linked immunosorbent assay (ELISA) kits manufactured by BioPorto Diagnostics A/S, Hellerup, Denmark.

Peripheral venous blood samples for serum creatinine analysis were collected prior to surgery, post-operatively after ICU admission and at 24, 48, 72 and 96 hours after surgery. Serum creatinine concentrations were measured using routine clinical analysis in our UK accredited clinical pathology laboratory.

Outcome measures

1. Acute Kidney Injury (AKI): Urinary NGAL levels were determined to assess predictive value for postoperative AKI. AKI was determined based on the definition of the international Kidney Disease Improving Global Outcomes (KDIGO) staging classification [23]. AKI is defined as an acute rise in serum creatinine from the preoperative value (obtained in the 30-day period before surgery) [22], where an acute rise is defined as values ≥26.5 µmol/L in the first 48 hours after surgery or ≥50% in the first 7 days after surgery. Briefly AKI was graded as follows based on changes in serum creatinine:

   Stage 1- serum creatinine increase of 1.5–1.9 times baseline value or an increase of ≥0.3 mg/dL (≥ 26.52 µmol/L).

   Stage 2 – serum creatinine increase 2.0–2.9 times baseline value.

Stage 3 – serum creatinine increase 3.0 times baseline, or increase in serum creatinine to ≥4.0 mg/dL (≥353.6 μmol/L).

The urine output criteria were not used as many patients required diuretics and/or haemofiltration the post-operative period.

2. Follow-up survival: Urinary NGAL levels were determined to assess predictive value for postoperative follow-up survival.

Statistical analysis

Statistical analysis was undertaken using SigmaPlot, SAS and StatsDirect software. Where there was non-Gaussian distribution, data was presented as median and range. Group differences were assessed using Kruskal Wallis, χ^2 or Mann-Whitney U tests where appropriate. Spearman correlation was used to determine correlations for non-normal distribution data or categorical variables. Non-normal distribution data was log-transformed to achieve normal distribution and analysed by Pearson correlation. A $p<0.05$ was considered statistically significant. Receiver Operating Characteristic (ROC) Curves were plotted to determine relationship between urinary NGAL and postoperative AKI. A Kaplan-Meier plot compared survival for patients with or without postoperative AKI and Cox proportional hazards regression model analysis was performed to identify factors with the greatest influence on survival.

Results

Patient characteristics

Patients' characteristics are summarised on Table 1. The median baseline urinary NGAL value in all patients was 23.00 (0.82-828.35) ng/mL. This was not found to be significantly different between current smokers 16.50 (4.41-725.36) ng/mL, ex-smokers 21.84 (1.44-828.35) or never-smoked patients 26.20 (0.82-655.46) ng/mL. There was also no difference detected between female and

male gender; 21.64 (0.82-806.53) and 21.84 (2.48-806.53) ng/ml respectively; or diabetic status; 18.61 (0.82-806.53) and 29.81 (2.48-828.25) ng/ml for none-diabetics and diabetics respectively (Table 1).

Postoperative outcomes

Following surgery 43% of the patients went on to develop AKI as determined by serum creatinine and RIFLE criteria. Of these, 26 were in RIFLE class 1, 21 in class 2 and 7 in class 3 as summarised on Table 2. There was no significant difference between cross-clamp time or on pump time between the two groups during surgery and there was no difference in incidence of postoperative AKI in the haemofiltration or non-haemofiltration groups. Baseline urinary NGAL concentration was not found to be significantly different between AKI and NKI patients and the median urinary NGAL concentration (ng/ml) was significantly raised ($p<0.0001$) in all patients, at all the time points, following surgery regardless of whether they went on to develop AKI. Urinary NGAL concentration was significantly different between the two groups at both 24 hours ($p=0.003$) and 48 hours ($p<0.0001$) post-surgery (figure 1). Those patients that went on to develop postoperative AKI had a significantly longer ICU stay ($p<0.0001$) and hospital stay (<0.0001) compared to those patients without kidney injury.

Correlations

Baseline and post-surgery urinary NGAL did not correlate with serum creatinine at any time point. 24 hour urinary NGAL concentrations weakly correlated with 24 hour ($r=0.290$, $p=0.001130$), 48 hour ($r=0.274$, $p=0.0020$), 72 hour ($r=0.289$, $p=0.00179$) and 96 hour ($r=0.296$, $p=0.00261$) serum creatinine. Whilst 48 hour urinary NGAL showed better correlations with 48 hour ($r=0.477$, $p<0.0001$), 72 hour ($r=0.448$, $p<0.0001$) and 96 hour ($r=0.463$, $p<0.0001$) serum creatinine (Figure 2).

There was no correlation between the cross-clamp time or bypass time and NGAL concentration at any time point. However, there was a correlation between urinary NGAL concentration at 48 hours post-surgery and duration of ICU stay hours (r=0.265, p=0.00285).

Receiver Operating Characteristic (ROC) Curves

ROC curves demonstrated that baseline urinary NGAL levels for patients with preoperative kidney impairment scheduled to undergo cardiopulmonary bypass surgery do not significantly predict AKI, as determined by serum creatinine. In contrast, post-operatively, urinary NGAL values at 48 hours showed the most significant predictive value for AKI with an AUC = 0.76, sensitivity =0.7037 and specificity = 0.6901 and a cut-off value of >156ng/ml (Figure 3).

Kaplan-Meier plot of 7-year follow-up survival

As shown on Figure 4 patients with postoperative AKI had significantly lower 7-year follow-up survival compared with those without AKI (P=0.013).

Cox proportional hazards regression model

Table 2 shows a summary of a Cox proportional hazards model indicating that only age, current or past smoking, Urinary NGAL levels at 48h > 156 ng/mL and postoperative antibiotics usage were strong hazards for 7-year follow-up survival. The results indicate that patients with urinary NGAL levels at 48 h >156ng/mL have an 80% greater risk of death during the 7-year follow-up period compared to those with NGAL at 48 h <156 ng/mL, although statistical significance was not reached. In addition, the results shown on Table 3 also show that urinary NGAL levels at 48 h >156ng/mL is a much stronger predictor of survival than acute kidney injury.

Discussion

This study has shown that for patients with preoperative kidney impairment postoperative urinary excretion of NGAL is elevated regardless of presence of AKI as assessed by KDIGO guidelines and RIFFLE Criteria [23]. Postoperatively only patients that develop AKI injury continue to present persistently elevated urinary NGAL at 24 hour time-point and beyond which may be indicative of late injury. The late injury may originate from an increased generation of free haemoglobin and iron through haemolysis are typically by-products of mechanical stresses that occur during cardiopulmonary bypass [24] mostly through cardiotomy suction, the duration of perfusion, occlusive roller pumps, turbulent flow in the oxygenator, and blood return through cell savers [24]. It has been shown previously [25] that the generation of free haemoglobin may contribute to oxidative stress and renal tubular injury [26]. Also low preoperative serum ferritin levels are associated with an increased risk for postoperative AKI [26]. Other potential contributors of late injury may include haemodilution [27-28] and the resultant decrease in oxygen carrying capacity [27-28]. Indeed, haemodilution (haematocrits ≤25%) is associated with an increased risk for renal injury as measured by changes in serum creatinine [29-30] which is thought to be due to the impairment of oxygen delivery to an already hypoxic renal medulla or to alterations in systemic inflammatory mediators caused by regional ischemia [30]. These are the potential mechanisms for the trigger of increased postoperative urinary NGAL excretion.

NGAL is a 25kDa protein that is predominantly expressed in neutrophils [31] and plays a role in iron metabolism [32] and as a bacteriostatic agent [33]. It is also expressed at very low levels in several human tissues and is induced in injured epithelial cells, including the liver, lung, colon and kidney. Analysis from animal studies has shown NGAL to be one of the most highly induced proteins in the kidney following injury, and the fact it can be easily detected in urine shortly afterwards makes it a promising non-invasive clinical biomarker [34]. Several studies have since taken place in different clinical settings with mixed results [19-23]. Following cardiac surgery the results of these studies have been particularly variable and there is limited information for the use of NGAL in those patients that already have kidney disease, as this is an exclusion criterion in many studies. As such, we

decided to investigate whether urinary NGAL is a predictive biomarker of AKI in high risk patients with preoperative renal impairment (eGFR <50ml/min) undergoing cardiac surgery.

We recruited 125 patients undergoing cardiac bypass surgery to our study, of which 43% developed AKI according to the KDIGO staging classification [23]. This incidence is higher than that commonly reported, but these were high risk patients who already had renal impairment, so a higher degree of kidney failure was to be expected. There was a significant increase in urinary NGAL in all patients at all time-points regardless of whether they went on to develop kidney injury. Despite this there was also a significant difference between the AKI and No-AKI groups at 24 and 48 hours. Furthermore, 24 and 48 hour urinary NGAL correlated with serum creatinine concentrations at later time points. Unlike, previous studies, there was no correlation between both cross-clamp time and time on bypass and urinary NGAL concentration following surgery. Despite a significant difference between the AKI and No-AKI groups at 24 and 48 hours the AUC-ROC was best at 48 hours post-surgery but still did not provide adequate prediction of AKI, as determined by serum creatinine, for clinical purposes.

The increase in urinary NGAL concentration in all patients regardless of time point or evolving preoperative AKI status suggests that an increase of NGAL from an extra-renal source is being observed. Animal models indicate that systemic NGAL filtered at the renal glomerulus is rapidly and efficiently reabsorbed by the proximal tubule (35). Whether or not this occurs in patients with chronic renal disease is unknown. NGAL is synthesised in other tissues besides the kidney and plays a key-role in the inflammatory response. Patients undergoing CPB are likely to have a systemic inflammatory response following surgery, activating neutrophils and causing them to release their granular contents, including NGAL, into the circulation. NGAL has also been identified within the macrophages of atherosclerotic plaques (36,37), a disruption of which may release further NGAL into the circulation. This may explain why the predictive value of urinary NGAL increases over time in this

subset of patient and as the 'noise' from systemic inflammation following surgery decreases in patients without AKI, it remains elevated in those who have developed post-operative AKI.

We also recognise in this study that assessing urinary NGAL against serum creatinine may not provide the best indicator of NGAL's use as a biomarker. Serum creatinine is a marker of kidney function, whilst NGAL is a biomarker of tubular injury and is derived from epithelial cells undergoing proliferation and regeneration as part of the repair process. These differences in their characteristics mean that directly comparing the two may under represent NGAL's value as a diagnostic marker of AKI. However, in the absence of another clinical biomarker of kidney injury the comparison between serum creatinine and NGAL concentrations will continue. Determination of urinary NGAL at 48 hours clinically could be important because levels >156ng/ml could be used to discriminate between the patients at high risk of developing further kidney injury, allowing for early within the first 24-48 hours postoperatively. An early intervention for AKI may impact on long term survival of patients. Recently, there has been a focus on the development of new types of predictive tests based on panels of biomarkers. Such an approach is now being tested clinically, the latest being the NEPHROCHECK test system and AKIRisk score based on measurement of a panel of biomarkers consisting of tissue inhibitor metalloproteinase-2 [38] and Insulin-like growth factor binding protein-7 [39], which are known to be involved in the responses to a variety of insults such as inflammation, oxidative stress, ultraviolet radiation, drugs and toxins [40-42]. We believe that the incorporation of urinary NGAL in the panel of biomarkers for acute kidney injury may in the future be useful in the enhancement of the specificity of such a test kit. The diagnostic performance of such a combination will require clarification on different patient populations before adoption of urinary NGAL into clinical practice becomes broadly acceptable.

We acknowledge that this study, did not determine changes in haematocrit (oxygen carrying capacity) and intraoperative hypotension across the study period, mechanisms described previously

[27-30] as potential causes of postoperative kidney injury and whether this may relate to the level of urinary NGAL excretion.

In conclusion, our study failed to show that baseline preoperative urinary NGAL predicts the onset and severity of AKI, based on KDIGO classifications, in patients with preoperative kidney disease. It is likely that the global systemic inflammatory response from bypass surgery triggers high levels of these markers such that the progression into kidney injury is masked by this event until 48 hours post-procedure where patients with AKI begin to emerge as having higher concentrations of urinary NGAL. It is highly probable that at 48 hours postoperative urinary NGAL not only indicate AKI but may also predict long term survival outcomes.

Conflict of interest:

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, et al (2005) Acute renal failure in critically ill patients: A multinational, multicenter study. J Am Med Assoc 294(7):813-818.
2. Mao H, Katz N, Ariyanon W, Blanca-Martos L, Adýbelli Z, Giuliani A, et al (2013).Cardiac surgery-associated acute kidney injury. CardioRenal Med 3(3):178-199.
3. Hansen MK, Gammelager H, Mikkelsen MM, Hjortdal VE, Layton JB, Johnsen SP, et al (2013) Post-operative acute kidney injury and five-year risk of death, myocardial infarction, and stroke among elective cardiac surgical patients: A cohort study. Crit Care 17(6).
4. Rosner MH, Okusa MD (2006) Acute kidney injury associated with cardiac surgery. Clin J Am Soc Nephrol. 1(1):19-32.
5. Thiele RH, Isbell JM, Rosner MH (2015) AKI associated with cardiac surgery. Clin J Am Soc Nephrol. 10:500-514.

6. Kiers HD, van den Boogaard M, Schoenmakers MCJ, van der Hoeven JG, van Swieten HA, Heemskerk S, et al. (2013) Comparison and clinical suitability of eight prediction models for cardiac surgery-related acute kidney injury. Nephrol Dial Transplant. 28:345-351.

7. Garg AX, Shehata N, McGuinness S, Whitlock R, Fergusson D, Wald R, et al. (2018) Risk of Acute Kidney Injury in Patients Randomized to a Restrictive Versus Liberal Approach to Red Blood Cell Transfusion in Cardiac Surgery: A Substudy Protocol of the Transfusion Requirements in Cardiac Surgery III Noninferiority Trial. Can J Kidney Health Dis. 5:2054358117749532. doi: 10.1177/2054358117749532. eCollection 2018.

8. Dasta JF, Kane-Gill SL, Durtschi AJ, Pathak DS, Kellum JA. (2008) Costs and outcomes of acute kidney injury (AKI) following cardiac surgery. Nephrol Dial Transplant. 23:1970-1974.

9. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates D (2005). Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol.;16:3365-3370.

10. Ishani A, Nelson D, Clothier B, Schult T, Nugent S, Greer N, et al (2011) The magnitude of acute serum creatinine increase after cardiac surgery and the risk of chronic kidney disease, progression of kidney disease, and death. Arch Intern Med. 171:226-233.

11. Machado MN,Nakazone MA, Maia LN (2014) Acute kidney injury based on KDIGO (Kidney Disease Improving Global Outcomes) criteria in patients with elevated baseline serum creatinine undergoing cardiac surgery. Rev Bras Cir Cardiovasc. 29(3):299-307.

12. Nickolas TL, Forster CS, Sise ME, Barasch N, Valle DS-, Viltard M, et al (2012) NGAL (Lcn2) monomer is associated with tubulointerstitial damage in chronic kidney disease. Kidney Int 82(6):718-722.

13. Han M, Li Y, Liu M, Li Y, Cong B. (2012) Renal neutrophil gelatinase associated lipocalin expression in lipopolysaccharide-induced acute kidney injury in the rat. BMC Nephrol 13(1).

14. Dent CL, Ma Q, Dastrala S, Bennett M, Mitsnefes MM, Barasch J, et al. (2007) Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: A prospective uncontrolled cohort study. Crit Care 11(6).

15. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, et al. (2005) Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 365(9466):1231-1238.

16. Friedrich MG, Bougioukas I, Kolle J, Bireta C, Jebran FA, Placzek M, et al. (2017) NGAL expression during cardiopulmonary bypass does not predict severity of postoperative acute kidney injury. BMC Nephrol 18(1):1-7.

17. Koyner JL, Bennett MR, Worcester EM, Ma Q, Raman J, Jeevanandam V, et al (2008) Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery. Kidney Int 74(8):1059-1069.

18. Wagener G, Jan M, Kim M, Mori K, Barasch JM, Sladen RN, et al (2006) Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. Anesthesiology 105(3):485-491.

19. Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT (2008) Urinary Neutrophil Gelatinase-Associated Lipocalin and Acute Kidney Injury After Cardiac Surgery. Am J Kidney Dis 52(3):425-433.

20. McMahon GM, Waikar SS (2013) Biomarkers in nephrology: Core Curriculum 2013. Am J Kidney Dis. 62:165-178.

21. Devarajan P (2010) Neutrophil gelatinase-associated lipocalin: a promising biomarker for human acute kidney injury. Biomark Med. 4: 265–280.

22. Matata BM, Scawn N, Morgan M, Shirley S, Kemp I, Richards S, et al (2015) A Single-Center Randomized Trial of Intraoperative Zero-Balanced Ultrafiltration During Cardiopulmonary Bypass for Patients With Impaired Kidney Function Undergoing Cardiac Surgery. Journal of Cardiothoracic and Vascular Anesthesia 29(5):1236–1247.

23. KDIGO Clinical Practice Guideline for Acute Kidney Injury Kidney International Supplements 2012. 2: 1. doi:10.1038/kisup.2012.1. http://www.kidney-international.org.

24. Wright G: (2001) Hemolysis during cardiopulmonary bypass: Update. Perfusion 16: 345–351,.

25. Baliga R, Ueda N, Waler PD, Shah SV. (1997) Oxidant mechanisms in toxic acute renal failure. Am J Kidney Dis 29: 465– 477.

26. Davis CL, Kausz AT, Zager RA, Kharasch ED, Cochran RP. (1999) Acute renal failure after cardiopulmonary bypass is related to decreased serum ferritin levels. J Am Soc Nephrol 10: 2396 –2402.

27. Messmer K (1975) Hemodilution. Surg Clin North Am 55: 659 – 678.

28. Shah D, Corson J, Karmody A, Leather R (1986) Effects of isovolemic hemodilution on abdominal aortic aneurysmectomy in high risk patients. Ann Vasc Surg 1: 50 –54.

29. Swaminathan M, Phillips-Bute BG, Conlon PJ, Smith PK, Newman MF, Stafford-Smith M (2003) The association of lowest hematocrit during cardiopulmonary bypass with acute renal injury after coronary artery bypass surgery. Ann Thorac Surg 76: 784 –792.

30. Karkouti K, Beattie WS, Wijeysundera DN, Rao V, Chan C, Dattilo KM, et al (2005) Hemodilution during cardiopulmonary bypass is an independent risk factor for acute renal failure in adult cardiac surgery. J Thorac Cardiovasc Surg 129: 391– 400.

31. Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N (1993) Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem 268(14):10425-10432.

32. Schmidt-Ott KM, Mori K, Jau YL, Kalandadze A, Cohen DJ, Devarajan P, et al (2007) Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 18(2):407-413.

33. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK (2002) The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell 10(5):1033-1043.

34. Mishra J, Qing MA, Prada A, Mitsnefes M, Zahedi K, Yang J, et al (2003) Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 14(10):2534-2543.

35. Mori K, Lee HT, Rapoport D, Drexler IR, Foster K, Yang J, et al (2005) Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest 115(3):610-621.

36. Hemdahl A-, Gabrielsen A, Zhu C, Eriksson P, Hedin U, Kastrup J, et al (2006) Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. Arterioscler Thromb Vasc Biol 26(1):136-142.

37. Eilenberg W, Stojkovic S, Piechota-Polanczyk A, Kaun C, Rauscher S, Gröger M, et al (2016) Neutrophil Gelatinase-Associated Lipocalin (NGAL) is Associated with Symptomatic Carotid Atherosclerosis and Drives Pro-inflammatory State in Vitro. Eur J Vasc Endovasc Surg;51(5):623-631.

38. Kashani K, Al-Khafaji A, Ardiles T, et al. (2013) Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. Crit Care.17:R25.

39. McCullough PA, Shaw AD, Haase M, et al. (2013) Diagnosis of Acute Kidney Injury Using Function al and Injury Biomarkers: Workgroup Statements from the Tenth Acute Dialysis Quality Initiative Consensus Conference. Contrib Nephrol.182:13-29.

40. Boonstra J, Post JA. (2004) Molecular events associated with reactive oxygen species and cell cyc le progression in mammalian cells. *Gene*. 337:1-13.

41. Seo DW, Li H, Qu CK, et al. (2006) Shp-1 mediates the antiproliferative activity of tissue inhibitor of metalloproteinase-2 in human microvascular endothelial cells. J Biol Chem. 281:3711-3721.

42. Price PN, Safirstein RL, Megyesi J. (2009) The cell cycle and acute kidney injury. Kidney Int. 76:60 4-613.

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Tables

Table 1: Patient characteristics

	No-AKI (n=71)	**AKI (n=54)**	**P-value**
Gender (female: male), n/n	38/33	20/34	0.073
Age, (median (range)) years	75 (59-85)	74 (54-87)	0.458
Intraoperative Haemofiltration	**33/71 (46.5)**	**28/54 (51.9)**	0.560
Post-operative Haemofiltration (n (%))	38 (54)	26 (48)	0.591
Procedure type (n (%))	CABG = 21 (29.6)	CABG = 16 (29.6)	0.999
	CABG + V = 16 (22.5)	CABG + V = 21 (38.9)	0.051
	MVR = 12 (16.9)	MVR = 3 (5.6)	0.093
	AVR = 22 (31)	AVR = 14 (25.9)	0.557
Diabetic, n (%)	20 (28.2)	22 (40.7)	0.900
Smokers, n (%)	Cigar =0(0)	Cigar = 1 (1.8)	0.437
	Current = 7 (9.9)	Current = 5 (9.3)	0.999
	Ex = 38 (53.5)	Ex= 31 (57.4)	0.719
	Never =26 (36.6)	Never = 17 (31.5)	0.574
EuroSCORE II	2.97 (0.69-16.32)	3.94 (0.67-35.28)	0.034
X-clamp time (min.)	75 (35-226)	78 (41-257)	0.980
On pump time (min.)	106 (54-258)	115 (66-380)	0.720
Baseline pre-operative urinary NGAL (ng/mL)	21.64 (0.82-828.35)	23.58 (1.44-655.46)	0.913
Post-operative urinary NGAL (ng/mL) on	83.65 (0.52-1254.50)	149.89 (2.58-1141.30)	0.421

admission to ICU			
24 hours Post-operative urinary NGAL (ng/mL)	143.24 (2.87-888.27)	237.61 (8.36-1154.37)	0.003
48 hours postoperative urinary NGAL (ng/mL)	93.92 (6.21-888.02)	288.93 (16.13-1048.00)	0.0001

ICU= Intensive care unit

Table 2: Perioperative Outcomes

Outcomes	**No-AKI (n=71)**	**AKI (n=54)**	**P-value**
In-hospital mortality	2 (2.8)	5 (9.3)	0.237
Stroke	4 (5.6)	2 (3.7)	0.698
Reoperation	9 (12.7)	4 (7.4)	0.339
Prolonged ventilation (>48 hrs)	2 (2.8)	6 (11.1)	0.075
Re-intubation	3 (4.2)	6 (11.1)	0.173
Deep sternal wound infection	0 (0)	0 (0)	-
Acute Kidney Injury, n (%) RIFLE 1 RIFLE 2 RIFLE 3	 0 0 0	 26 21 7	 <0.0001
ICU length of stay (days)	3 (2-26)	7 (2-93)	<0.0001
Post-operative length of stay (days)	9 (3-26)	15 (5-93)	<0.0001

ICU= Intensive care unit

Table 3: Cox proportional hazards analysis for factors influencing 7-year survival

Variable	Hazard ratio	95% confidence interval	*P-value*
Age (years)	1.056	1.011-1.102	0.010
Female sex	1.602	0.842-3.050	0.150
Diabetes	1.233	0.626-2.431	0.540
Current smoker	3.271	1.080-9.902	0.040
Ex-smoker	1.941	0.945-3.989	0.070
NGAL (48hr) > 156 ng/mL	1.799	0.961-3.367	0.070
Acute kidney injury	1.395	0.752-2.587	0.290
Chest infection	1.549	0.730-3.286	0.250
Postoperative antibiotics usage	2.480	1.334-4.608	0.004
ICU length of stay (hours)	1.000	0.999-1.001	0.810

Postoperative antibiotic mean antibiotics given on suspicion of infection

Figure legends

Figure 1: Urinary NGAL concentrations in patients with no acute kidney injury (NKI) versus acute kidney injury (AKI). Data is expressed as ng/ml. *P<0.05, **P<0.01, ***P<0.001. Statistical significance was determined using Kruskall-Wallis test.

Figure 2: Correlation between 48 hour NGAL and serum creatinine concentration. Urinary NGAL concentration is expressed as ng/ml and serum creatinine as mg/L. Correlation was undertaken using Spearman Correlation test.

Figure 3: Receiver operating characteristic curves for urinary NGAL in patients who developed post-operative AKI. Urinary NGAL concentrations were measured at baseline, post-surgery, and at 24 and 48 hours following surgery. At baseline P=0.9127, post-surgery P=0.4209, 24 hours after surgery P=0.0032 and 48 hours following surgery P<0.0001.

Figure 4: Analysis of association between long term survival and presence or absence of acute kidney injury using a Kaplan Meier plot.