Urinary Neutrophil Gelatinase Associated Lipocalins (NGALs) predict acute kidney injury post liver transplant.

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Abstract

Acute Kidney Injury, a common complication of liver transplant, is associated with a significant increase in the risk of morbidity, mortality and graft loss. Current diagnostic criteria leaves a delay in diagnosis allowing further potential irreversible damage. Early biomarkers of renal injury are of clinical importance and Neutrophil Gelatinase Associated Lipocalins (NGALs) and Syndecan-1 were investigated.

Methods

AKI was defined according to the Acute Kidney Injury Network criteria. Urine and blood samples were collected pre-operatively, immediately post-op and 24 hours post reperfusion to allow measurement of NGAL and Syndecan-1 levels.

Results

13 of 27 patients developed an AKI. Patients who developed AKI had significantly higher peak transaminases. Urinary NGAL, plasma NGAL and Syndecan-1 levels were significantly elevated in all patients post reperfusion. Urinary NGAL levels immediately post-op were significantly higher in patients who developed an AKI than those that didn't [1319ng/ml vs 46.56ng/ml, p=<0.001]. ROC curves were performed and urinary NGAL levels immediately post-op were an excellent biomarker for AKI with an area under the curve of 0.948 (0.847-1.00).

Discussion

Urinary NGAL levels measured immediately post-op accurately predict the development of AKI and their incorporation into clinical practice could allow early protocols to be developed to treat post transplant AKI.

Introduction

Acute Kidney Injury (AKI) is a common complication post Orthotopic Liver Transplant (OLT). The diagnosis of AKI centres around serial changes in serum creatinine levels over 48 hours and AKI is classified according to the Acute Kidney Injury Network (AKIN) criteria [1] or the Risk, Injury, Failure, Loss of kidney function, End-stage kidney disease (RIFLE) [2] criteria. In general hospital inpatients, the development of AKI is associated with increased morbidity, mortality and use of hospital resources [3]. A recent study performed at the Royal Free Hospital showed that early detection of AKI could improve outcomes [4].

The incidence of AKI post liver transplant in the literature is extremely varied with incidence rates of between 14% and 97% [5–8]. A recent review of liver transplant outcomes at our unit found a post transplant AKI incidence of 50% with 24% of patients requiring Renal Replacement Therapy (RRT) [8]. The development of AKI post liver transplant is associated with increased morbidity, mortality and graft loss [6], increased length of stay in both the intensive care department and hospital post transplant [5] and the development of chronic kidney disease (CKD) [7].The physiological abnormalities associated with cirrhosis and end stage liver failure and the physiological changes occurring as a result of the transplant procedure make creatinine measurements in this group of patients unreliable [9]. The identification of reliable biomarkers of AKI in these patients is therefore of significant importance. Many post transplant immunosuppressive regimens and analgesia include nephrotoxic medications including Tacrolimus [10] and non steroidal anti-inflammatory medications [11]. Early identification of AKI post transplant may allow for early avoidance of nephrotoxins and early intervention of the renal physicians. The development of early and reliable biomarkers for AKI is therefore of key importance.

Neutrophil Gelatinase Associated Lipocalin (NGAL) is a 25kDa protein that is rapidly released by damaged nephrons [12,13] and has been shown to accurately diagnose AKI in patients undergoing major cardiac surgery [14,15] and poor graft function post renal transplant [16]. A recent systematic review identified several small studies that have investigated the role of urinary and plasma NGAL levels in diagnosing AKI post liver transplant with varying results [17]. There were several limitations to these studies including significant variability within each trial as to the time of sample

collection. Furthermore no study commented on NGAL levels in patients who progressed to require RRT which is arguably a more important endpoint. Further studies with robust and early collection protocols are therefore required.

Syndecan-1 is shed following vascular injury resulting in disruption of the glycocalyx. Ischaemia Reperfusion (IR) injury in liver transplant patients is associated with significant injury to the microvascular network which may be a major factor leading to renal injury [18]. The role of Syndecan-1 in predicting AKI post liver transplant remains to be fully elucidated [19].

The aim of this study was to investigate for biomarkers of AKI post liver transplant.

Methods:

Patients

Serial urine and blood samples were collected and analysed from 27 patients undergoing liver transplant at the Royal Free Hospital, London. Patients who were recruited to this study were a consecutive group of patients involved in a randomized controlled pilot trial of recipient limb preconditioning, the Remote Ischaemic Preconditioning in Orthotopic Liver Transplantation (RIPCOLT) trial, the results of which have previously been published [20]. The study conformed with the declaration of Helsinki and written informed consent was obtained from all patients. The trial protocol was approved by the National Ethics Service (11/H0720/4) and the Royal Free Hospital/University College London ethical board (8191) and was published [21]. The trial was registered in Clinical trials.gov (NCT00796588). Inclusion criteria for the RIPCOLT trial were adult patients undergoing first elective deceased donor liver transplant. Exclusion criteria included age <18, re-transplantation, grafts transported on the organox machine perfusion system, transplantation on a super-urgent basis and absence of informed consent.

Liver transplantation

Grafts were identified and retrieved through the dedicated UK National Organ Retrieval Service (NORS) according to national standards of organ retrieval from deceased donors [22] (NHSBT). Following aortic cannulation all grafts were perfused in situ with cold University of Wisconsin (UW) solution (Bridge to Life, Chicago) at a maximum pressure of 200mmHg. On removal the grafts were further flushed with ice cold UW solution on the back bench via the hepatic artery, portal vein and the bile duct. The grafts were then sterile packaged in cold UW solution and transported to the recipient hospital on ice.

The recipients were monitored intra-operatively via arterial and central venous catheters with availability of trans-oesophageal echo as required. Implantation of the liver graft was performed by standard piggy-back and caval replacement techniques. Veno-venous bypass was not employed in any patient randomized in this trial. Grafts were flushed with 500-1000mls warm 4.5% human albumin solution (Bio Products Laboratory) via the portal vein immediately prior to blood re-perfusion to remove residual UW solution and waste material accumulated during cold ischaemia. 1g of

methylprednisolone (Pharmacia) was given intravenously during the anhepatic phase as part of standard anaesthetic protocol.

Post operative management

Post-operatively all patients were managed in the intensive care unit.
Haemoglobin levels were maintained below 10g/L. Platelets and fresh frozen plasma were administered if there was a coagulopathy associated with active blood loss. Patients were routinely started on subcutaneous thromboprophylaxis on the first post operative day. All patients underwent a Doppler ultrasound scan of the liver vessels on the first, third and fifth post-operative day. Daily blood tests included clotting profiles, renal function, bilirubin and serum liver enzymes.
Patients were extubated on the first post-operative day unless there was a clinical need for ongoing respiratory support and triple therapy immunosuppression was commenced on day 1 post-operatively. If there was evidence of early renal impairment, monoclonal antibody therapy was given in place of triple therapy immunosuppression. The need for renal replacement therapy was decided following clinical review and review of clinical biochemistry.

Sample collection and analysis

Peripheral venous blood was collected on the day of admission for liver transplant and daily in the first post-operative week. Blood samples were measured for haemoglobin levels, serum transaminases and bilirubin levels, urea and creatinine levels and coagulation profiles. Further peripheral venous blood samples were collected at 1 and 3 months post transplant to allow creatinine levels to be measured. Creatinine ratios were calculated by dividing the creatinine levels at 1 and 3 months by the pre-operative value to allow for changes in renal function to be identified.
To obtain the freshest sample of urine that reflected the time point at which the sample was collected, urine samples were collected directly from the side port in the foley catheter, and not from the catheter reservoir at 3 different time points: At baseline following induction of anaesthesia but before skin incision; immediately following skin closure; and at 24 hours post transplant. Urine samples were immediately centrifuged (10 minutes, 1000g, 20^0C) and then frozen to -80^0C and stored at this temperature until analysis. Five mls of peripheral venous blood was collected at baseline, 2 hours post reperfusion, and 24 hours post reperfusion in

plasma containers (BD, UK). Blood samples were immediately centrifuged (10 minutes, 1000g, 20^0C) to pellet the cells. The plasma was immediately alliqoted into 150µl samples and frozen to -80^0C and stored at that temperature until analysis. Samples were thawed and urinary NGAL levels (Biolegend, UK), plasma NGAL (Biolegend, UK) and Syndecan-1 levels (Abcam, UK) were analysed by ELISA on commercially available kits according to manufacturers guidelines.

Acute Kidney Injury

AKI was defined according to the AKIN network score as an absolute rise in creatinine levels by 26.4µmo/L or a rise from baseline in serum creatinine levels by 150% within the first 48 hours post transplant [1]. Patients who underwent renal replacement therapy were automatically classified as developing an AKI.

Peri-operative Urine Volumes

The anaesthetic and intensive care charts were reviewed retrospectively and the hourly urine volumes were collected both during the intra-operative period and in the first 48 hours of the ITU stay or till discharge to the ward if earlier. The patent's weight was measured on admission for liver transplant. Hourly urine volumes were therefore converted to urine output/kg/hour.

Statistics

Continuous variables were described as mean (± standard deviation) or median (inter-quartile range) as appropriate and differences between the groups were analysed by T-tests or Mann-Whitney U tests as appropriate. Dichotomous variables were described as a percentage of the total and differences between the groups were identified by Chi-squared test. ROC curves, and likelihood ratios were calculated to identify cut off values at predicting AKI and need for RRT. Statistics were performed on SPSS 21 (IBM) and Prism 5 (Graphpad, USA).

Results

Samples were collected and analysed from 27 adult patients undergoing first elective liver transplant surgery at the Royal Free Hospital, London. Donor, recipient and transplant variables are contained within table 1. Thirteen patients (48%) developed an AKI post liver transplant and 7 patients (26%) required RRT. There was no significant difference at baseline between patients who developed an AKI and those that did not (Table 1).

Donor, Transplant and Recipient variables associated with AKI

No donor variable was associated with the development of an AKI (Table 2). Patients who developed an AKI had a higher median time taken to perform the vascular anastomoses however this was not statistically significant (Table 1) Patients who developed an AKI had significantly higher peak AST and ALT levels (Table 1). AST levels on day 3, a good marker of graft and patient outcome [23], were higher but this was not statistically significant (Table 1). Recipient MELD and UKELD were higher in patients that developed an AKI but this was not statistically significant

Patients with AKI had lower urine output than those that did not suffer an AKI

Although patients who developed an AKI had lower peri-operative urine volumes, this was not significant. Urine volumes between the groups were similar in the first 6 hours post transplant. By 6 hours post-op patients with an AKI had significantly lower urine volumes than those that did not. By 24 hours post op this difference had disappeared (Table 3). A ROC found the intra-operative urine output to have an AUROC of 0.731 (0.530-0.931).

Patients with AKI showed a trend towards poorer long-term renal function

Creatinine ratios in patients with an AKI were higher than in those without an AKI at both 1 month [1.2(±0.6) vs 1.0(±0.2), p=0.2] and at 3 months [1.4(±0.6) vs 1.3(±0.3), p=0.4] but this was not significant.

Patients who developed an AKI had higher urinary NGAL levels immediately post liver transplant

No patient was unable to provide a urine sample for analysis due to anuria however due to clinical time constraints, urine samples at time of abdominal closure were not collected in 2 patients. The mean time post portal vein reperfusion till collection of urine samples at abdominal closure for analysis was 178(±46) minutes. Urinary NGAL levels were significantly elevated post reperfusion [160.67(30.74-1319.64)ng/ml vs 56.29(14.1-116.8)ng/ml, p=0.018] and remained elevated at 24 hours post transplant [90.91(43.78-744.77)ng/ml]. Patients who developed an AKI had higher urinary NGAL levels at baseline than those that did not (Table 2). Patients who developed an AKI had significantly higher urinary NGAL levels at abdominal closure (Figure 1) and at 24 hours post reperfusion (Table 2). Patients who required RRT had significantly higher urinary NGAL levels at 24 hours post reperfusion (Table 4). Patients who required RRT had higher urinary NGAL levels at time of abdominal closure however this failed to reach statistical significance (Table 4).

Among AKI patients, urinary NGAL levels do not predict which patients will require RRT

When patients who did not develop an AKI were removed from the analysis there was no significant difference between patients with an AKI that required RRT and those with an AKI that did not [1344.55(1230.98-2230.28)ng/ml vs 838.63(420.75-2216.94)ng/ml, p=0.368].

Plasma NGAL levels do not predict AKI

Median plasma NGAL levels were significantly elevated 2 hours post reperfusion when compared to baseline [921.62(587.96-1248.07)ng/ml vs 246(162.24-406.97)ng/ml, p<0.001]. There was no significant difference in plasma NGAL levels of patients that developed an AKI and those that did not, either at 2 hours or 24 hours post reperfusion (Table 2). Similarly there was no significant difference between plasma NGAL levels of patients that required RRT and those that did not, either at 2 hours or 24 hours post reperfusion (Table 4).

Patients who developed an AKI had higher plasma Syndecan-1 levels at 24 hours post reperfusion

Syndecan-1 levels were significantly elevated at 2 hours post reperfusion when compared to baseline [462(415.45-553.89)pg/ml vs 73.0(54.54-169.32)pg/ml, $p<0.001$]. Patients who developed an AKI had significantly higher syndecan-1 levels at baseline and at 24 hours post-reperfusion (Table 2). There was no significant difference in Syndecan-1 levels of patients that developed an AKI and those that did not at 2 hours post reperfusion (Table 2). Patients who required RRT had significantly lower Syndecan-1 levels at 2 hours post reperfusion (Table 4). By 24 hours post reperfusion patients who required RRT had higher Syndecan-1 levels but this was not significant (Table 4).

Urinary NGAL immediately post liver transplant is an excellent predictor of the development of AKI

We produced ROC curves to identify the cutoff values of urinary NGAL levels at time of abdominal closure and at 24 hours post reperfusion in identifying patients who would develop AKI and those that would require RRT.

Analysis of urinary NGAL levels at time of abdominal closure gave an Area Under the Curve of 0.948 (0.847-1) identifying it as an excellent predictor of AKI and an AUC of 0.75 (0.532-0.968) identifying it as a fair predictor of the need for RRT (Figure 2). Analysis of urinary NGAL levels at 24 hours post reperfusion gave an AUC of 0.792 (0.614-0.969) identifying it as a fair predictor of AKI and an AUC of 0.812 (0.624-1) identifying it as a good predictor of the need for RRT (Figure 2). Likelihood ratios were then calculated.

To predict AKI, a urinary NGAL level of 170ng/ml or greater at time of abdominal closure was associated with a likelihood ratio of 14.08 and a positive predictive value (PPV) of 92% and a negative predictive value (NPV) of 100%.

To predict the need for RRT, a urinary NGAL level of 1018ng/ml or greater at time of abdominal closure was associated with a likelihood ratio of 4 and a PPV of 50% and a NPV of 89%. A urinary NGAL level of 102ng/ml 24 hours post reperfusion was associated with a likelihood ratio of 3.3 and a PPV of 55% and a NPV of 94%.

Plasma Syndecan-1 levels at 24 hours post reperfusion are a good indicator of the development of AKI

We produced ROC curves to identify the cutoff values of plasma Syndecan-1 levels at 24 hours in identifying patients who have an AKI and those that will require RRT. The AUC was 0.836 (0.644-1.00) identifying it as a good biomarker of AKI and was 0.735 (0.492-0.977) identifying it as a fair marker for the requirement of RRT (Figure 3).

Remote Ischaemic Preconditioning has no effect on AKI, NGAL or Syndecan-1 levels

As previously published, Remote Ischaemic Preconditioning (RIPC) had no effect on the incidence on AKI or need for RRT post liver transplant [20]. There was no significant difference in urinary NGAL levels of patients who underwent RIPC and controls either at time of abdominal closure ($p=0.501$) or at 24 hours post reperfusion ($p=0.150$). There was similarly no significant difference in Syndecan-1 levels at 24 hours ($p=0.089$)

Discussion:

This study would suggest that urinary NGAL levels can be used as an early biomarker of AKI in patients undergoing liver transplant. Furthermore it shows promise at predicting patients that will require renal replacement therapy post-operatively.

In this prospective study, 48% of patients developed an AKI and 26% required RRT. A larger observational study performed at our unit over 1 year including 116 patients undergoing liver transplant identified an incidence of AKI of 50% and 24% required RRT [8]. This would suggest that this smaller cohort is representative of our patients in general.

Patients who developed an AKI had significantly higher post-operative AST and ALT levels in keeping with more severe graft IR injury [24]. The degree of graft IR injury has previously been associated with the development of post transplant AKI [8,24]. Interestingly there was no significant difference between the groups regarding length of cold ischaemic time suggesting that strategies to reduce the post reperfusion inflammatory response may result in reduced incidence of AKI with its associated increase in morbidity and mortality [8].

In this study there was no significant increase in the incidence of AKI following implantation of a DCD graft. This may reflect the small number of DCD donors in this study as the use of a DCD graft has previously been associated with an increased incidence of AKI secondary to IR injury [24].

Previous studies investigating the role of urinary NGALs post liver transplant were limited by the inability to collect urine samples from patients who became anuric during the transplant [25]. In this study, we were able to collect fresh urine samples from all patients. In only 2 patients there was no sampling at the end of surgery due to a processing error.

This study has clearly shown that urinary NGAL levels measured on completion of the liver transplant can accurately predict patients who will progress to develop AKI. Only one patient with a urinary NGAL level of above 170ng/ml did not develop an AKI. This was a small woman of 48kg who experienced a rise in creatinine levels of 25μmol/L. Although she did not meet the criteria for AKI (creatinine rise of

26.4μmol/L), she clearly suffered a renal injury. The AURC of 0.948 identifies urinary NGAL levels as an excellent predictor of AKI. This AURC is better than reported in any other study investigating urinary NGALs post liver transplant (0.5-0.83). This likely reflects the standardized and early collection protocol used in this study. In our study fresh urine samples were collected at the time of abdominal closure before the patient left the operating theatre and the time from graft reperfusion to sample collection was less that 3 hours. It is known that following tubular injury, NGALs are released early and peak in the urine within 4 hours [26]. The collection protocol in previous studies varied widely with samples collected at differing time points within 24 hours of arrival to ITU. Sick patients post liver transplant with multi organ dysfunction will require a longer period of stabilization prior to study sample collection. This may result in a bias of samples from unstable patients being collected later at which point NGAL levels may have peaked at 4 hours and be dropping. Samples from this study collected at 24 hours had an AUC of 0.792 which is similar to previous studies and likely reflects that earlier post transplant NGAL measures are a better diagnostic test.

Although patients who developed an AKI had a lower intra-operative urine output than those that did not, this was not significant and differences in urine volumes between the groups only became significantly after 6 hours post-op. The measurement of urine volumes would therefore result in a delay of diagnosis over urinary NGALs.

Plasma as well as urinary NGAL levels were measured in this study. Although plasma NGAL levels increased post reperfusion the increase did not correlate with the development of AKI. Although renal NGAL production and release is significantly upregulated following AKI, NGAL is not specific to the renal tissue and can be released by the heart, activated neutrophils and other ischaemic tissues [27]. NGALs are freely absorbed and reabsorbed by the kidneys and only accumulate in the urine following tubular injury as this prevents re-absorption. The raised plasma levels seen in all patients following graft reperfusion may reflect both the hepatic IR injury and neutrophil activation seen post reperfusion.

Syndecan-1 plasma levels again were also elevated following graft reperfusion. Levels at 24 hours post reperfusion were higher in those who developed AKI. This

likely reflects the degree of graft IR injury, its associated microvascular disturbance and resulting damage to the endothelial glycocalyx. One study has previously investigated the role of Syndecan-1 at predicting AKI post transplant. Patients who developed AKI grade 2 or 3 had significantly higher Syndecan-1 levels than those with no renal injury or those that developed an AKI grade 1 [19]. Although plasma Syndecan-1 levels can identify AKI at 24 hours post reperfusion, urinary NGAL levels can predict AKI much earlier and therefore are of more clinical use.

Interestingly patients who developed an AKI had higher baseline Syndecan-1 and urinary NGAL levels. This would suggest that these patients have risk factors predisposing them to AKI although there were no differences in baseline characteristics between patients that developed an AKI and those that did not. The measurement of Syndecan-1 levels pre-operatively may therefore be of interest in identifying patients at risk of developing an AKI however this would need to be proved in a larger cohort.

Patient who developed an AKI as defined by urinary NGALs had poorer renal function as determined by their serum creatinine levels at 30 and 90 days post-op compared to baseline. This is in keeping with previous studies that have shown that the development of AKI is associated with long term renal dysfunction and chronic renal failure [7]. Although this was not significant this may reflect the small numbers in this study.

Studies aimed at reducing AKI post liver transplant are hampered by the inclusion of many patients who will not develop renal injury. The identification of patients who suffer from peri-transplant renal injury by measurement of urinary NGALs at the end of the liver transplant procedure would allow the effective design of smaller and less expensive controlled trials aimed at early interventions to treat AKI post liver transplant.

There is evidence to suggest that the use of goal directed post-operative fluid therapy may reduce the incidence of AKI in patients post coronary artery bypass surgery [28]. Some trials, however have suggested that goal directed therapy (GDT) is not associated with a reduction in AKI post major abdominal surgery [29]. Its role in

reducing the incidence of AKI post liver transplant remains to be elucidated but is the subject of a current trial [30]. A randomized controlled trial of goal directed therapy in patients with raised NGAL levels would be an attractive method of identifying whether GDT post transplant reduces AKI in patients with evidence of renal injury. However biomarker validation would be required before this trial design could be justified.

Three studies have identified that early initiation of RRT in patients with severe AKI either post cardiac surgery [31], major trauma [32] or in patients admitted to ITU [33] is associated with a significant reduction in mortality, ITU and hospital stay and improved long-term renal function. In our institution, all patients undergoing liver transplant surgery have central venous lines inserted pre-operatively for RRT in case it is required. Validation of this study results would allow for a well designed randomized controlled trial investigating the effect of early RRT in patients with evidence of renal injury as identified by raised urinary NGAL levels at the end of surgery.

The trend towards increased utilization of DCD grafts [34,35] will result in an increasing incidence of post transplant AKI. Current strategies to treat AKI remain limited. Early identification of patients who have suffered peri-operative renal injury would allow for the avoidance of nephrotoxic medications where possible, the incorporation of reno-protective immunosuppressive regimes including monoclonal antibodies in the post-operative period and the optimisation of fluid balance. The results of this study raise new opportunities in the early treatment of AKI by using urinary NGALs to identify patients with peri-operative renal injury before it has been identified on clinical or biochemical grounds. One limitation to this strategy is that the method of NGAL measurement in this study by standard ELISA took 7 hours to perform. However there are commercial point of care tests available which would allow NGAL measurements within 1 hour [36]. Further work is now required to validate the use of these assays in liver transplant patients. This would also allow these results to be validated in another cohort of patients.

In conclusion, by using a strict and easily reproducible collection protocol for measuring urinary NGAL levels, patients who suffer from peri-operative renal injury

and who develop AKI following liver transplant can be identified with a high level of accuracy by the end of the operative procedure. This will allow for resources to be allocated to improving outcomes for these patients and help design trials aimed at reducing AKI post liver transplant.

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Variables	AKI	Non-AKI	p
Donor			
Sex (M/F)	9/4	11/3	
Age	53 (37-59)	44 (18-51)	0.49
DCD	3	2	0.63
DRI	1.62 (1.43-1.79)	1.61 (1.42-2.03)	0.83
Recipient			
Age	55 (53-59)	59 (49-64)	0.36
Sex (M/F)	12/1	11/3	
MELD	15 (14-16)	14 (9-16)	0.28
UKELD	54 (53-57)	52 (48-56)	0.14
Pre-op creatinine	81 (67-98)	75 (70-86)	0.4
Transplant			
Cold ischaemic time (mins)	449 (393-492)	601 (451-633)	0.03
Anastomosis time (mins)	44 (39-50)	39 (33-44)	0.06
Operative time (mins)	423 (387-505)	445 (420-475)	0.87
RCC transfusion (units)	4 (2-4)	2 (0-4)	0.2
Piggyback	8	6	
Peak AST (iU/L)	2821 (1609-3217)	1152 (541-2190)	0.006
Peak ALT (iU/L)	1606 (729-2749)	746 (481-1090)	0.03
Day 3 AST (iU/L)	418 (103-491)	183 (95-337)	0.17
EAD	8	7	0.44

Table 1: Donor, recipient and peri-transplant variables of patients who developed an AKI compared to those that did not. Values are median (IQR).

Abv: DCD – Donor following Cardiac Death, DRI – Donor Risk Index, MELD – Model for End Stage Liver Disease, UKELD – United Kingdom Model for End Stage Liver Disease, RCC – Red Cell Concentrate, AST – Aspartate Transferase, ALT – Alanine Transferase, EAD – Early Allograft Dysfunction.

	Time points	AKI	Non-AKI	p
Urinary NGALs (ng/ml)	Baseline	71(29-220)	30(14-72)	0.09
	Abdominal closure	1319(783-2480)	46(16.-104)	<0.001
	24 hours post reperfusion	471 (83-1172)	49(35-94)	0.01
Plasma NGALs (ng/ml)	Baseline	291(150-407)	234(188-327)	0.94
	2 hours post reperfusion	1134 (788-1225)	892(588-1259)	0.87
	24 hours post reperfusion	448(396-600)	641(320-698)	0.35
Plasma Syndecan-1 (pg/ml)	Baseline	117(73-339)	58(38-77)	0.02
	2 hours post reperfusion	44(382-534)	531(415-561)	0.47
	24 hours post reperfusion	355(319-399)	173(114-234)	0.01

Table 2: Biomarkers between patients that developed an Acute Kidney Injury (AKI) and those that did not. Values are median (IQR). Significance determined by Mann Whitney U tests.

Time-point	AKI	No AKI	p value
Intra-op	1.26 (±0.86)	1.8 (±0.7)	0.09
1-6 hours post-op	1.1 (0.5-1.4)	1.3 (1.2-2.4)	0.12
6-12 hours post-op	0.4 (0.2-0.7)	0.95 (0.5-1.5)	0.01
12-24 hours post-op	0.43 (±0.4)	0.76 (±0.34)	0.03
24-48 hours post-op	0.56 (±0.43)	0.69 (±0.24)	0.34

Table 3: Average hourly urine outputs between patients with Acute Kidney Injury (AKI) and not.

	Time points	RRT	No RRT	p
Urinary NGALs (ng/ml)	Baseline	95(70-216)	31(10-76)	0.06
	Abdominal closure	1320(1142-1369)	114(23-839)	0.1
	24 hours post reperfusion	745(104-1786)	63(35-197)	0.02
Plasma NGALs (ng/ml)	Baseline	407(333-466)	228(161-306)	0.06
	2 hours post reperfusion	802(294-1225)	977(633-1254)	0.65
	24 hours post reperfusion	455(404-599)	601(381-698)	0.5
Plasma Syndecan-1 (pg/ml)	Baseline	121(69-356)	62(52-77)	0.15
	2 hours post reperfusion	436(270-444)	549(456-571)	0.01
	24 hours post reperfusion	356(286-399)	208(117-350)	0.09

Table 4: Biomarkers between patients that required Renal Replacement TherapyRRT and those that did not. Values are median (IQR). Significance determined by Mann Whitney U tests.

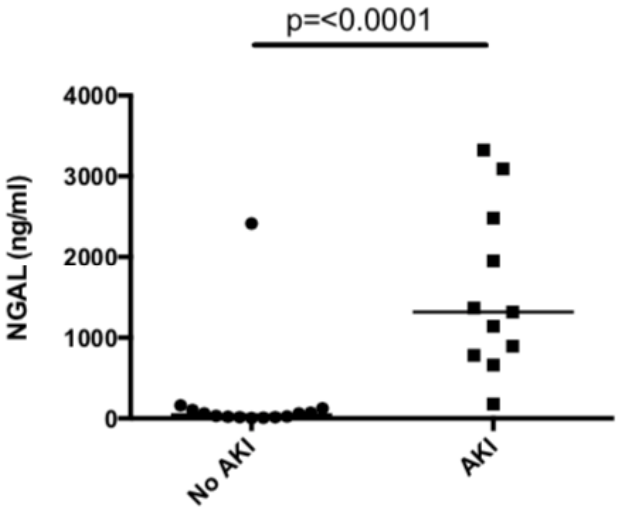

Figure 1: Patients who develop AKI have significantly higher median urinary

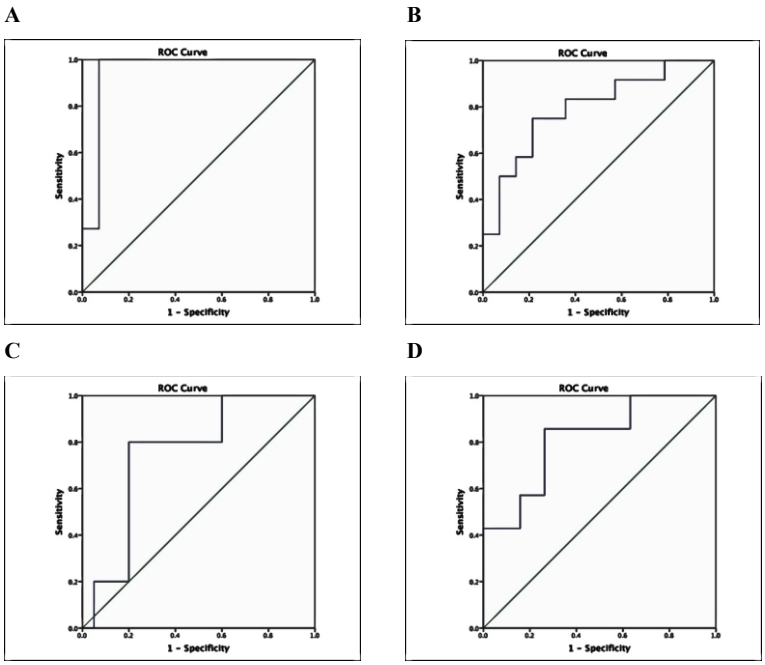

Figure 2

A: Urinary NGAL levels measured at abdominal closure at detecting AKI [AUC 0.948 (0.847-1.00)]

B: Urinary NGAL levels measured at 24 hours at detecting AKI [AUC 0.792 (0.614-0.969)]

C: Urinary NGAL levels measured at abdominal closure at predicting RRT [AUC 0.750 (0.532-0.968)]

D: Urinary NGAL levels measured at 24 hours at predicting RRT [AUC 0.812 (0.624-1.00)]

A **B**

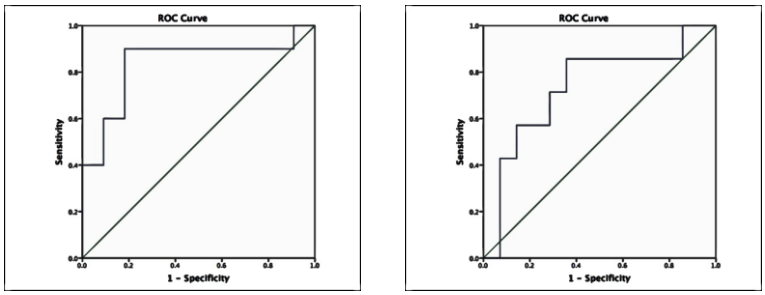

Figure 3

A: Plasma Syndecan-1 levels measured at 24 hours at detecting AKI [AUC 0.836 (0.644-1.0)]

B: Plasma Syndecan-1 levels measured at 24 hours at predicting RRT [AUC 0.735 (0.492-0.977)]