



# Comparison of Novel Early Biomarkers of Acute Kidney Injury (AKI) to predict the Course and Severity of AKI Developing after Cardiac Surgery



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## Objective

Evaluate the utility of urinary biomarkers Cystatin C (CyC), Neutrophil Gelatinase Associated Lipocalin (NGAL), Kidney Injury Molecule-1 (KIM-1), Hepatocyte Growth Factor (HGF),  $\pi$ -Glutathione-S-Transferase ( $\pi$ -GST) and  $\alpha$ -GST as markers of Acute Kidney Injury severity, at the time of clinical creatinine increase, following adult cardiac surgery.

## Background

Acute Kidney Injury (AKI) is common following cardiothoracic (CT) surgery occurring in 7-42% of patients<sup>1,2</sup>. Small changes in serum creatinine have been shown to correlate with increased morbidity and mortality, following CT surgery<sup>3-5</sup>. Patients developing severe AKI requiring Renal Replacement Therapy (RRT) after CT surgery have a greatly increased in-hospital mortality (63%) compared to those with non-dialyzed AKI (19%), or stable renal function (0.9%)<sup>1</sup>. Emerging biomarkers of AKI are being investigated to facilitate earlier diagnosis and treatment of AKI. The role of these "early" biomarkers in the diagnostic evaluation AKI (at the time of creatinine increase) remains unclear.

Cystatin C (CyC), a 13kDa cysteine protease inhibitor, is an effective marker for GFR<sup>6</sup>. Recent data suggest the utility of urine CyC in the early diagnosis of AKI in adult CT surgery patients<sup>7</sup>. NGAL (25kDa), a member of the lipocalin family, is markedly upregulated in the early post-ischemic mouse and rat kidney<sup>8</sup>. Multiple investigators have shown that urine NGAL increases earlier than creatinine in AKI following cardiac surgery<sup>7, 9, 10</sup>. KIM-1 is a transmembrane protein that is highly over-expressed in dedifferentiated proximal tubule cells after ischemic or nephrotoxic AKI<sup>11</sup>. KIM-1 has been shown to increase earlier than serum creatinine in pediatric AKI following cardiac surgery<sup>12</sup>. HGF has been shown to be elevated in the setting of AKI<sup>13</sup>.  $\alpha$ -GST and  $\pi$ -GST, specific to proximal and distal tubule of the human kidney respectively have been shown to be elevated in the setting of AKI<sup>14</sup>.

## Methods

**Subjects** - All patients undergoing elective CT surgery at the University of Chicago between August 2005 and the present were screened and approached for enrollment. Patients were excluded if they met any of the following criteria:

- Pre-existing ESRD (on RRT) or Renal Transplant
- Age <18 years old

• Unstable renal function ( $\Delta$  Serum Creatinine  $\geq 0.2$  mg/dl in the last 2 months or Oliguria defined as < 400 ml / day)

**Procedures** - Subjects were prospectively followed with blood and urine collection at predetermined timepoints throughout their hospitalization:

- At study enrollment
- 4 times on the day of the surgery
- Daily for 5 days

**Sample Handling** - Urine samples (spun and unspun) were stored at -80° C; the urine was centrifuged at 4000 rpm for 10 minutes.

**Assays** - Creatinine was measured via the Jaffé Method  
Cystatin C was measured in the University of Chicago using the Human Cystatin C ELISA kit (BioVendor, LLC - Candler N.C., USA).  
NGAL was measured at the Cincinnati Children's Hospital using the NGAL ELISA kit (HYB211-05, AntibodyShop, Genotf, Denmark)  
Kim-1 and HGF protein were measured at Brigham and Womens Hospital using Microsphere-based Luminex xMAP™ technology with monoclonal antibodies in the Vaidya laboratory.  
 $\alpha$ -GST and  $\pi$ -GST were measured via commercially available enzyme immuno-assay (Argutus Medical, Dublin Ireland)

## Results

**Data Analysis** - The primary endpoint AKI was defined as a  $\geq 50\%$  or 0.3 mg/dL increase in the plasma creatinine (AKIN Stage 1) from the pre-operative baseline or the need for renal replacement therapy within 72 hours of surgery. Early Post-op Max was defined as the maximum value from the first 3 post operative timepoints (PostCPB, ICU arrive and 6hr ICU; within the first 6 post-op hours).

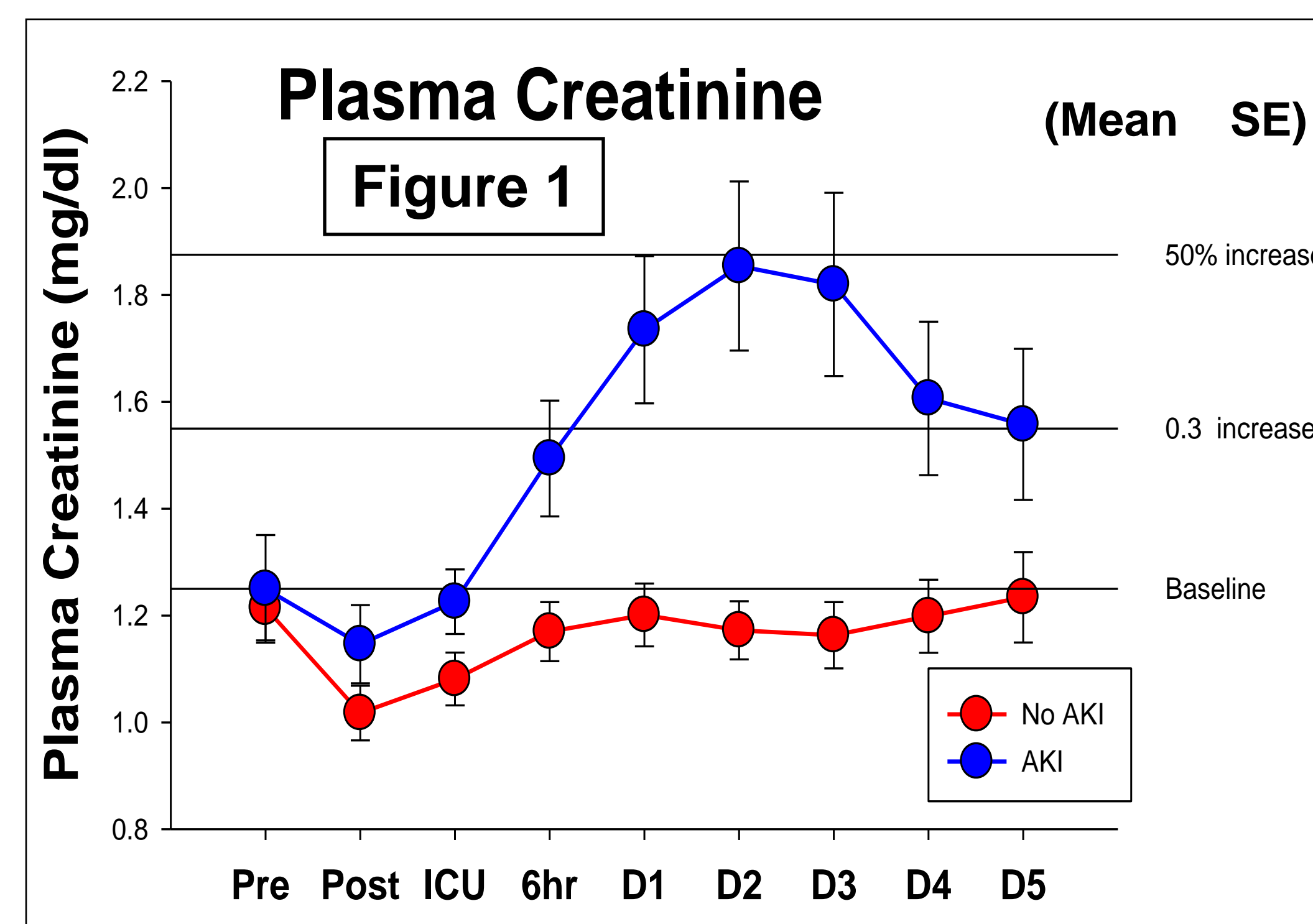


Figure 1 shows the Plasma Creatinine over time for those subjects with (n=46) and without AKI (n=77). Note that AKI can not be diagnosed until at least Day 1 or 2.

Table 1: Clinical Characteristics of University of Chicago Cardiac Surgery Study Subjects (n=123)

	No AKI (n=77)	AKIN Stage 1-2 (n=37)	AKIN Stage 3 (n=9)	
Age (median (IQR))	68 (56.5-77)	72 (61 - 81)	72 (59 -79.3)	
Sex	M / F (%)	66.2/ 33.8	62.2/37.8	66.7/ 33.3
Race	Caucasian Afr. American Other	54 18 5	32 4 1	6 3 0
Hispanic	N (%)	5 (6.5)	2 (5.4)	1 (11.1)
Baseline GFR (MDRD)	ml/min/1.73m <sup>2</sup> Mean SE	72.3 3.2	73.1 5.4	64.4 9.4
Baseline Creatinine†	mg/dL Median (IQR)	1.04 (0.87- 1.33)	1.09 (0.90 - 1.20)	1.40 (1.20 - 1.67) †
Diabetes Mellitus	N (%)	21 (27.2)	10 (27.0)	3 (33.3)
COPD	N (%)	19 (27.5)	7 (18.9)	3 (33.3)
Ejection Fraction	N. (Mean SE)	59 (44.4 2.2)	29 (49.5 3.2)	5 (51.0 4.0)

Preoperative Meds				
Beta Blockers	N (%)	32 (41.6)	9 (24.3)	5 (55.5)
ACE-I/ARB	N (%)	23 (29.9)	11 (29.7)	4 (44.4)
Calcium Channel Blockers	N (%)	12 (15.6)	4 (14.8)	2 (22.2)
Diuretics	N (%)	28 (36.4)	8 (21.6)	6 (66.6)
Statins	N (%)	27 (35.1)	11 (29.7)	5 (55.5)

Operative Factors				
Surgery Type	CABG alone Valve alone CABG + Valve CABG + Other Valve + Other Other	24 10 20 3 17 3	8 11 10 0 6 2	2 1 5 0 1 0

Previous CT surgery	N= (%)	18 (23.4)	5 (13.5)	2 (22.2)
Cardiopulmonary Bypass Time #	Minutes (Median (IQR))	162 (126-213)	160.5 (128 - 214)	250.5 (182 - 345.5)
Off-pump Surgery	N=	8	3	1
Cross Clamp Time	Minutes Mean SE	126.2 5.6	129.3 10.9	156.1 16.6
No Cross Clamp	N =	14	3	1

Outcomes				
ICU Days , ‡	Median (IQR)	3 (2 - 5)	3 (2 - 5)	9 (5.75-16) ‡
Post-Op Hospital Days #	Mean SE	7 (6 - 9.3)	7 (6 - 10.3)	19 (8.5 - 30.3) #
In Hospital Mortality , †	N =	0	1	4

No AKI vs. AKI p < 0.05; Stage 3 vs. All others † p < 0.05; # p < 0.01; ‡ p = 0.001. All other p=NS

Table 2: Biomarker as Predictors of AKI Severity at time of Creatinine Increase

median (25-75% IQR)	Stage 1 or 2 N=37	Stage 3 N=9	AUC (95%CI)	P value
FENa %	0.312 (0.010 - 0.869)	2.12 (0.23 - 2.395)	0.671 (0.469 - 0.874)	NS
FE Urea %	34.5 (14.7 - 38.4)	33.4 (19.2 - 60.8)	0.512 (0.254 - 0.769)	NS
CyC (mg/L)	.114 (0.027-0.23)	.272 (0.22 - 0.72)	0.751 (0.563 - 0.939)	0.021
CyC / Creat mg/g	0.101 (0.040 - 0.32)	0.725 (0.51 - 1.17)	0.768 (0.565- 0.972)	0.019
NGAL	51.6 (17.7 - 301)	483 (341 - 2300)	0.818 (0.647 - 0.987)	0.010
NGAL/Creat ng/mg	43.9 (14.1-167.5)	573 (508 - 8536)	0.798 (0.593 - 0.998)	0.015
KIM1 (pg/ml)	672.9 (407.2 - 2492)	743.4 (575.8-938.8)	0.485 (0.292 - 0.678)	NS
KIM1 / Creat ng/mg	0.987 (0.480 - 1.74)	1.59 (1.02 - 1.83)	0.647 (0.457 - 0.837)	NS
HGF (pg/ml)	164.3 (56.2 - 524.5)	209.8 (123.7 - 1344)	0.645 (0.444 - 0.846)	NS
HGF /Creat (ng/mg)	0.148 (0.062 - 0.763)	0.601 (0.161 - 3.675)	0.677 (0.477 - 0.876)	NS
$\alpha$ -GST ( $\mu$ g/L)	1.197 (0.776 - 2.79)	3.604 (0.380 - 21.3)	0.597 (0.287 - 0.907)	NS
$\alpha$ -GST / Creat (ng/mg)	1.45 (0.844 - 3.48)	4.84 (0.483 - 1002)	0.603 (0.303 - 0.903)	NS
$\pi$ - GST ( $\mu$ g/L)	3.4 (3.4 - 8.44)	67.75 (13.1 - 30,015)	0.839 (0.667 - 0.998)	0.006
$\pi$ -GST /Creat (ng/mg)	3.19 (2.28 - 10.8)	92.1 (16.9 - 1317)	0.867 (0.735 - 0.999)	0.003

Table 3 - AUC for ROC Curves for Prediction of Stage 1 Early AKI (n=46)

Future Development Stage 1 AKI	AUC (95% CI)	P value
Cystatin C ICU Arrival value (mg/g)	0.659 (0.549 - 0.769)	0.005
Cystatin C 6 Hour ICU value (mg/g)	0.639 (0.525 - 0.751)	0.019
Cystatin C Early Post-op Max (mg/g)	0.681 (0.577 - 0.785)	0.001
NGAL ICU Arrival value (ng/mg)	0.685 (0.572 - 0.799)	0.002
NGAL 6 Hour ICU value (ng/mg)	0.713 (0.605 - 0.822)	< 0.001
NGAL Early Post-op Max (ng/mg)	0.674 (0.561 - 0.788)	0.004
KIM-1 ICU Arrival value (ng/mg)	0.584 (0.473 - 0.695)	NS
KIM-1 6 Hour ICU value (ng/mg)	0.672 (0.563 - 0.782)	0.004
KIM-1 Early Post-op Max (ng/mg)	0.679 (0.578 - 0.780)	0.0014
HGF ICU Arrival value (ng/mg)	0.541 (0.424 - 0.657)	NS
HGF 6 Hour ICU value (ng/mg)	0.465 (0.347 - 0.582)	NS
HGF Early Post-op Max (ng/mg)	0.627 (0.521 - 0.734)	0.02
$\alpha$ -GST ICU Arrival value (ng/mg)	0.591 (0.471 - 0.710)	NS
$\alpha$ -GST Hour ICU value (ng/mg)	0.475 (0.347 - 0.603)	NS
$\alpha$ -GST Early Post-op Max (ng/mg)	0.631 (0.519 - 0.744)	0.02
$\pi$ -GST ICU Arrival value (ng/mg)	0.605 (0.459 - 0.750)	NS
$\pi$ -GST Hour ICU value (ng/mg)	0.566 (0.447 - 0.686)	NS
$\pi$ -GST Early Post-op Max (ng/mg)	0.597 (0.480 - 0.714)	NS

Table 4 - AUC for ROC Curves for Prediction of AKIN Stage 3- Severe AKI

Future Development Stage 3	AUC (95% CI)	P value
Cystatin C ICU Arrival value (mg/g)	0.818 (0.672 - 0.965)	0.002
Cystatin C 6 Hour ICU value (mg/g)	0.773 (0.540 - 0.992)	0.02
Cystatin C Early Post-op Max(mg/g)	0.847 (0.716 - 0.977)	0.0006
NGAL ICU Arrival value (ng/mg)	0.820 (0.679 - 0.961)	0.002
NGAL 6 Hour ICU value (ng/mg)	0.878 (0.737 - 0.998)	< 0.001
NGAL Early Post-op Max (ng/mg)	0.816 (0.663 - 0.696)	0.003
KIM-1 ICU Arrival value (ng/mg)	0.665 (0.388 - 0.943)	NS
KIM-1 6 Hour ICU value (ng/mg)	0.533 (0.286 - 0.780)	NS
KIM-1 Early Post-op Max (ng/mg)	0.710 (0.506 - 0.914)	0.04
HGF ICU Arrival value (ng/mg)	0.640 (0.386 - 0.888)	NS
HGF 6 Hour ICU value (ng/mg)	0.680 (0.477 - 0.884)	NS
HGF Early Post-op Max (ng/mg)	0.666 (0.461 - 0.870)	NS
$\alpha$ -GST ICU Arrival value (ng/mg)	0.599 (0.346 - 0.851)	NS
$\alpha$ -GST Hour ICU value (ng/mg)	0.674 (0.379 - 0.968)	NS
$\alpha$ -GST Early Post-op Max (ng/mg)	0.631 (0.398 - 0.865)	NS
$\pi$ -GST ICU Arrival value (ng/mg)	0.688 (0.473 - 0.904)	0.063
$\pi$ -GST Hour ICU value (ng/mg)	0.762 (0.550 - 0.974)	0.021
$\pi$ -GST Early Post-op Max (ng/mg)	0.740 (0.550 - 0.930)	0.018

## Conclusions

At the time of the clinical diagnosis of AKI, as measured by AKIN Stage 1,  $\pi$ -GST, CyC, and NGAL all serve as markers of AKI severity. These markers can discriminate between those who will peak at Stage 3 AKI and all others. These markers may play a role in the diagnostic and clinical management of AKI following CT surgery at the time of plasma creatinine increase.

Urine CyC, NGAL, KIM-1, HGF and  $\alpha$ -GST all serve as early biomarkers of  $\geq$ Stage 1 AKI following adult cardiac surgery

Urine CyC, NGAL, KIM-1 and  $\pi$  - GST serve as early biomarkers of severe AKI (Stage 3)

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