

EXPEDIENCY OF MARKERS FOR EARLY DETECTION OF ACUTE KIDNEY INJURY SEQUELAE TO TYPE 2 DIABETES MELLITUS

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ABSTRACT

Objective: Estimation of Cystatin C (Cys C), traditional markers, inflammatory, and endothelial cell activation markers can identify subjects who are at increased risk for future acute kidney injury (AKI) after diabetes.

Methods: A total of 210 subjects, having 70 subjects in each group between the age group of 45-75 years were enrolled in our study.

Results: Body mass index (BMI), obesity index, waist circumference, and waist-hip ratio higher in Group III and Group II compared to Group I with a significant $p < 0.001$. All the biochemical parameters were significantly higher in Group III compared to Group I and Group II with a narrow difference between Group III and Group II. Serum Cys C was significantly correlated with creatinine and NO. Whereas serum creatinine (SCr) shows strong positive correlation with BMI, fasting blood sugar, HbA1c, NO, and high-sensitivity C-reactive protein. Estimated glomerular filtration rate (eGFR) and triglycerides show inverse relation to creatinine with a significant p-value. The serum Cys C showed an area under the curve (AUC) of 0.950 with a cutoff value of 1.06, SCr with an AUC of 0.617, and eGFR with AUC of 0.588.

Conclusion: Elevated levels of biomarker Cys C, SCr, and albumin-creatinine ratio are predictors of AKI in the setting of diabetes. Baseline inflammatory and endothelial activation markers may also be useful for predicting future risk of AKI in diabetes mellitus. Hopefully, the advent of new biomarkers will help defining the kidney at risk rather than relying simply on creatinine. To date, none of the new AKI biomarkers have undergone a similar rigorous assessment, but the current progress will hopefully lead to success and ultimately to improvement in patient outcome.

Keywords: Type 2 diabetes mellitus, Acute kidney injury, Biomarkers, Inflammation, Endothelium.

INTRODUCTION

Diabetes mellitus (DM), a clinical syndrome characterized by hyperglycemia, due to impaired insulin secretion and/or its function is associated with kidney dysfunction and face higher risks of morbidity and mortality [1]. Diabetes is one of the risk factors for developing acute kidney injury (AKI), a protean syndrome of varied severity, defined by rapid decline in glomerular filtration rate (GFR) resulting in retention of metabolic waste products such as urea, creatinine, and electrolytes associated with dysregulation of fluid and electrolyte balance [2]. Oxidative stress in diabetes is responsible for endothelial dysfunction and release of inflammatory markers such as cytokines from the damaged renal tissue. A number of different inflammatory cells and soluble mediators have been shown to be necessary for renal damage and loss of glomerular filtration. The proximal events leading to damage of renal tubular epithelial cells likely start in the microvasculature [3]. Inflammation within the kidneys are due to metabolic and hemodynamic factors with locally released cytokines such as tumor necrosis factor- α , interleukin-6 forming renal lesion through several mechanisms, including direct cellular injury, alteration of the glomerular protein permeability barrier, and development of intrarenal inflammatory damage resulting in diabetic nephropathy [4]. Studies conducted by Carmen and Navarro shown that inflammation and stress increases in critically ill patients with AKI [5]. Early diagnosis of AKI remains a challenge; unfortunately, serum creatinine (SCr) is a late marker for reduced GFR, which limits its ability to detect AKI promptly [5].

Newer biomarkers help the clinicians in detecting kidney injury earlier and also guide them for future therapy. The currently employed, traditional markers of AKI in the blood (creatinine and urea nitrogen) are insensitive and lag, they are not specific for any given disease process [6].

Cystatin C (Cys C) is a glycoprotein with a small size (13 KDa), has a positive charge at physiological pH and also an endogenous cysteine proteinase inhibitor. It is a member of the family of proteins that play a major role in the intracellular catabolism of various peptides and proteins [7,8]. Cys C is produced by all nucleated cells in the human body, synthesized at a relatively constant rate, and released into plasma. CysC is more than 99% filtered by the glomeruli, with no significant protein binding. CysC is believed to be neither actively secreted into the tubular lumen nor reabsorbed into the plasma. After filtration, CysC is normally completely reabsorbed by proximal renal tubular epithelial cells, through megalin receptor-induced endocytosis, and catabolized [9]. It is well documented that Cys C is less affected by age, race, and muscle mass in the general population. It is also associated strongly with cardiovascular mortality compared to SCr [2]. The aim of this study is to find the "expediency of markers for early detection of AKI sequelae to type 2 DM (T2DM)."

Objectives

1. Estimate and compare anthropometric, inflammatory, oxidative stress, and other biochemical parameters in clinically proven healthy controls (Group I), T2DM without AKI (Group II), and T2DM with AKI (Group III)
2. Correlate Cys C and SCr in Group II and III with anthropometric and biochemical parameters and also to know the severity of kidney injury
3. Receiver operating characteristic (ROC) analysis of Cys C, SCr, and estimated GFR (eGFR) to predict better biomarker for AKI.

METHODS

The study design is prospective case-control study conducted at RL Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Constituent of Sri Devaraj Urs Academy of Higher

Education and Research, Kolar, Karnataka. The study was approved by the Institutional Ethical Committee, and informed consent was obtained from all the study subjects. A total of 210 subjects in the age group 45-75 years of either gender were included and grouped into:

- Group I: Clinically proven healthy controls (n=70)*
- Group II: T2DM subjects without AKI (n=70)*
- Group III: T2DM subjects with AKI (n=70)*.

*AKI was diagnosed by risk, injury, failure, loss, and end-stage (RIFLE) criteria and AKI network: SCr levels >1.5 mg/dl and urine output (UO) <0.5 ml/kg/hr for >6 hrs (R), UO <0.5 ml/kg/hr for >12 hrs (I), UO <0.3 ml/kg/hr × 24 hrs or Anuria × 12 hrs [F] [10].

Exclusion criteria

- Patients with DM predisposed to radio contrast-induced nephrotoxicity
- DM with type 4 renal tubular acidosis (hyper-renin hypoaldosteronism, patients on drugs known to cause proteinuria/albuminuria)
- Nondiabetic with proteinuria/albuminuria
- Patients with hepatobiliary disorders leading to proteinuria/albuminuria
- Gestational DM
- Patients already diagnosed with diabetic nephropathy and who underwent dialysis.

Anthropometric measurements and biochemical estimations

Anthropometric measurements were measured and calculated using standard methods. Right arm's blood pressure was measured in sitting position using mercury sphygmomanometer. Blood sample was collected in the plain tubes after overnight fasting; all the parameters were estimated by the following methods. Fasting blood sugar (FBS) by glucose oxidase-peroxidase method [11], serum uric acid by uricase method [11], triglycerides by enzymatic GPO-PAP method [11], total cholesterol by enzymatic CHOD/PAP method [11], and HDLc was done by CHOD/PAP enzymatic method [11] by Dry Chemistry Analyzer, Vitros 250. HbA1c: High-performance liquid chromatography method, BIO-RAD D10, Nitric oxide: Modified Griess method [12], high-sensitivity C-reactive protein (hs-CRP): Latex turbidimetry [13], malondialdehyde (MDA): Thiobarbituric acid method [14], and Vitamin C: 2, 4-dinitro phenyl hydrazine method [15], these parameters were measured by spectrophotometry. The enzyme-linked immunosorbent assay kit (BioVendor, Brno, Czech Republic) was used for the determination of serum Cys C [16]. Calculations for LDLc: Friedwald's formula [11] and eGFR: Modification of diet in renal disease equation [17]. Urine albumin was measured by spot urine sample by dipstick method and for those with high values suspected to be at risk, the microalbumin was estimated using commercially available kit method and autoanalyzer. Urinary albumin-creatinine ratio (ACR) was calculated by dividing the urinary albumin concentration in microgram by the urinary creatinine concentration in milligram [17].

Statistical analysis

Was carried out by one-way analysis of variance, SPSS version 16.0, p<0.05 was considered statistically significant. Correlation of parameters within the groups was done by Pearson's formula. ROC analysis using MedCalc software.

RESULTS

Mean and standard deviation of anthropometric and physiological variables of 210 subjects enrolled in this study were shown in Table 1.

All the biochemical parameters were significantly higher in Group III compared to Group I and Group II with a narrow difference between Group III and Group II (Table 2).

Associated variables to serum Cys C, Creatinine, NO, hs-CRP, and HbA1c in diabetes with and without AKI were shown in Figs. 1-5. Serum Cys C was significantly correlated with creatinine (r=0.365, p<0.001) and NO (r=0.335, p<0.001). However, body mass index (BMI), FBS, HbA1c, ACR, vitamin C, eGFR, and MDA were positively correlated with no significant p-value. Whereas SCr shows strong positive correlation with BMI (r=0.537, p<0.05), FBS (r=0.557, p<0.001), HbA1c (r=0.319, p<0.001), NO (r=0.411, p<0.001), and hs-CRP (r=0.241, p<0.001). eGFR (r=-0.758, p<0.001) and triglycerides (r=-0.435, p<0.001) show inverse relation to creatinine with a significant p-value.

ROC analysis was performed to define the diagnostic profile of Cys C, Creatinine, and eGFR in T2DM subjects with and without AKI (Fig. 6). The serum Cys C showed an area under the curve (AUC) of 0.950 (95% CI, 0.867-0.989) with a cutoff value of 1.06 (sensitivity, 81.0%; specificity, 87.1%). SCr with an AUC of 0.617 (95% CI, 0.489-0.734) and eGFR with AUC of 0.588 (95% CI, 0.460-0.708).

DISCUSSION

AKI, a heterogeneous syndrome, has a crude mortality rate of 50% in patients with DM with or without complications [18].

In our study, we observed significant increase in FBS and HbA1c in Group I versus Group II, Group II versus Group III, and Group I versus Group III similar to the studies conducted by Shimada *et al.*, where they pointed out that mesangial expansion is the major lesion of diabetic nephropathy resulting in renal dysfunction in type 2 diabetes [19]. Glucose toxicity is a primary cause of glomerular injury in patients with diabetic nephropathy. Prolonged elevations in blood glucose levels result in the formation of glycation end products, which interfere with normal collagen turnover and promote vessel permeability, matrix accumulation, and the formation of adhesion molecules. Glucose can also bind reversibly and eventually irreversibly to proteins in the kidneys to form advanced glycation end products (AGEs). AGEs can form

Table 1: Mean and standard deviation of anthropometric and physiological variables of three groups

Parameters	Mean±SD			ANOVA F-value with significance
	Group I	Group II	Group III	
Age (years)	62.22±7.27	54.02±10.50	58.41±9.81	13.26; p<0.05*
Height (cm)	155.77±7.79	162.56±8.48	160.81±6.51	14.89; p<0.001**
Weight (kg)	55.05±9.57	69.68±10.16	67.71±8.96	48.05; p<0.001**
BMI (kg/m ²)	22.91±3.31	26.13±3.53	26.8±3.45	39.21; p<0.001**
OBI	55.77±7.79	62.55±8.48	60.81±6.51	14.89; p<0.001**
WC (cm)	92.38±8.67	97.40±5.01	96.60±0.78	10.04; p<0.001**
HC (cm)	96.71±5.92	95.47±5.08	96.11±5.09	0.62; p=0.53
WHR	0.95±0.06	1.02±0.04	1.00±0.06	0.85; p<0.001**
SBP (mmHg)	124±5.33	126±5.04	128±9.85	1.44; p=0.23
DBP (mmHg)	80±4.62	80±4.21	84±5.14	3.61; p<0.001**

*p<0.05 significant, **p<0.001 highly significant. Group I: Nondiabetes (clinically proven healthy controls), Group II: Diabetes without AKI, Group III: Diabetes with AKI. BMI: Body mass index, OBI: Obesity index, WC: Waist circumference, HC: Hip circumference, WHR: Waist-hip ratio, DBP: Diastolic blood pressure, SBP: Systolic blood pressure, SD: Standard deviation, AKI: Acute kidney injury

Table 2: Mean and standard deviation of biochemical parameters of three groups

Parameters	Mean±SD			ANOVA F-value with significance
	Group I	Group II	Group III	
FBS (mg/dl)	91.60±14.44	187.99±24.61	249.91±32.18	9.96; p<0.001**
HbA1c (%)	6.31±1.47	9.06±2.35	10.88±2.08	7.33; p<0.001**
SCr (mg/dl)	0.6±0.14	0.94±0.31	2.40±0.46	29.55; p<0.001**
Uric acid (mg/dl)	4.92±1.00	5.85±1.17	7.09±1.08	5.40; p<0.001**
Urine albumin	221.05±100.82	269.78±112.73	705.38±123.14	11.08; p<0.001**
Urine creatinine	90.96±29.91	59.96±16.07	56.06±17.30	2.22; p<0.05*
ACR	10.44±3.58	15.11±5.23	35.11±9.74	4.51; p<0.05*
eGFR (ml/min/1.73 m ²)	120.36±15.46	84.67±16.55	50.78±7.05	0.46; p<0.05*
TC (mg/dl)	100.69±15.53	181.81±34.81	175.87±26.13	201.03; p<0.001**
TG (mg/dl)	94.97±14.15	232.83±52.66	185.03±20.01	224.89; p<0.05*
HDLc (mg/dl)	39.85±7.39	45.15±6.90	48.65±7.15	12.57; p<0.001**
LDLc (mg/dl)	79.82±17.76	180.23±37.81	170.22±22.82	268.04; p<0.001**
Cys C (ng/ml)	2080.9±1233.19	4734.7±2241.01	5273.8±2534.82	12.10; p<0.001**
NO (μM/L)	54.06±18.59	34.48±7.44	28.78±4.89	86.84; p<0.001**
hs-CRP (mg/L)	2.12±0.95	1.98±0.45	0.94±1.12	0.074; p<0.05*
MDA (nmol/ml)	2.25±0.92	8.48±2.50	9.90±2.68	12.84; p<0.05*
Vitamin C (mg/dl)	1.18±0.56	0.42±0.13	0.35±0.37	0.84; p<0.001**

*p<0.05 significant, **p<0.001 highly significant. Group I: Non-Diabetes (clinically proven healthy controls), Group II: Diabetes without AKI, Group III: Diabetes with AKI, FBS: Fasting blood sugar, HbA1c: Glycated hemoglobin, SCr: Serum creatinine, ACR: Albumin creatinine ratio, TC: Total cholesterol, TG: Triglycerides, HDLc: High density lipoproteins, LDLc: Low density lipoproteins, Cys C: Cystatin C, NO: Nitric oxide, hs-CRP: High sensitive C-reactive protein, MDA: Malondialdehyde, SD: Standard deviation, AKI: Acute kidney injury

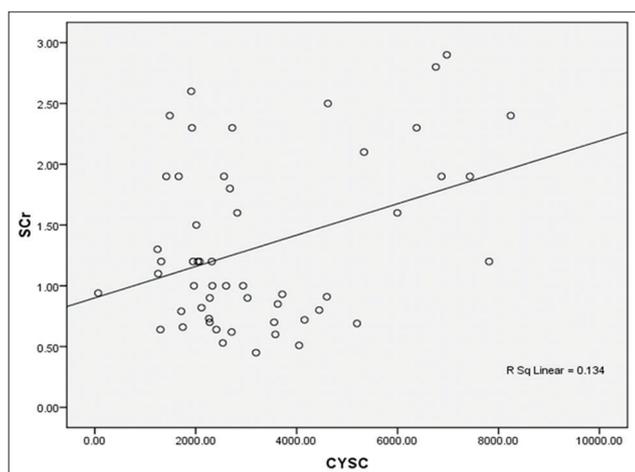


Fig. 1: Cystatin C versus serum creatinine

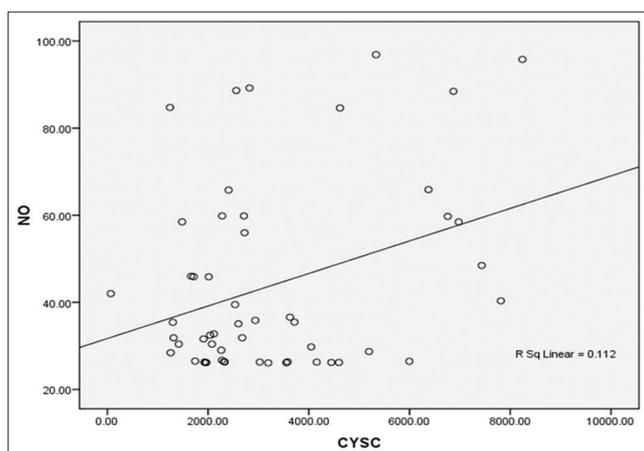


Fig.2: Cystatin C versus NO

complex cross-links over years of hyperglycemia and can contribute to renal damage. It has been noted that mediators of proliferation and expansion including platelet-derived growth factor, transforming

growth factor-beta, and vascular endothelial growth factor are also elevated in diabetic nephropathy [19].

Uric acid, the final oxidation product of purine metabolism excreted by kidneys, was significantly increased in Group II and III compared with Group I. Compared to the studies conducted by Shashidhar *et al.*, we also observed elevated serum uric acid levels in patients with reduced GFR [17]. In our study, eGFR was decreased in AKI patients with diabetes when compared with diabetic patients without AKI. It is well noted that angiotensin II increases efferent arteriolar pressure and plays a key role in the autoregulation of renal blood flow (RBF) and GFR. It is also known that prolonged inappropriate increase in angiotensin II lead to decrease in RBF and GFR and also release cytokines and growth factors [20]. In our study, around 50% of Group III patients (n=30) were on angiotensin II inhibitors.

Triglyceride levels were almost doubled in Group II compared to Group I indicating that increased blood sugar in Group II might have contributed to the elevated triglycerides. To our surprise, we could observe that the HDL levels are significantly elevated in Group III and Group II when compared to Group I, may be because of the factors: (a) lifestyle modification, (b) strict diet control, (c) statins, (d) awareness of the sequelae. The LDL levels were significantly elevated in Group III and Group II more than the double compared to Group I. Similar findings were observed in the study conducted by Munilakshmi *et al.* [11].

NO levels were almost half in Group III compared to Group I with a significant p-value. The reason may be because of narrowing of blood vessel or other contributing factors. To our surprise, the hs-CRP level is almost half in Group III compared to Group I may be because these people were on treatment and constant monitoring. MDA levels were 4-5 times elevated in Group II and Group III, respectively, when compared to Group I. Vitamin C levels were <50% in Group III compared to Group I but almost similar values with a narrow difference was observed between Group III and Group II. Our findings were consistent with the study done by Pou *et al.* [21]. Oxidative stress in diabetes is responsible for endothelial dysfunction releasing inflammatory markers such as cytokines from the damaged renal tissue. Alterations in endothelial dysfunction cause elevated expression and plasma levels of vasoconstrictors such as angiotensin II and endothelin-1 with increased expression of adhesion molecules and enhanced adhesion of platelets and monocytes to vascular endothelium releasing NO and reduced NO

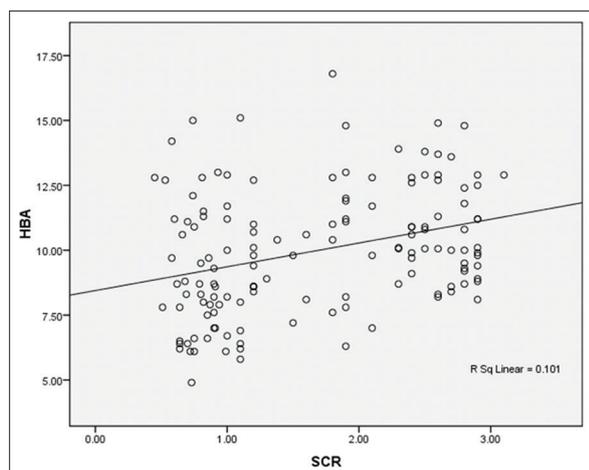


Fig. 3: Cystatin C versus NO

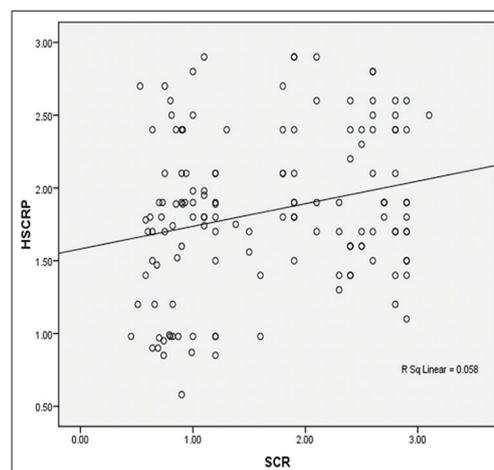


Fig. 5: Serum creatinine versus high-sensitivity C-reactive protein

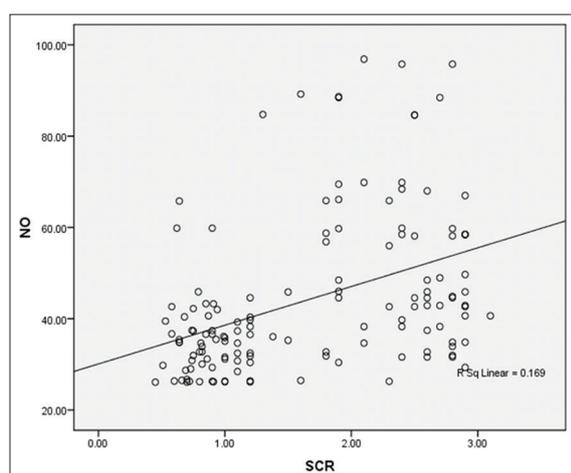


Fig. 4: Serum creatinine versus NO

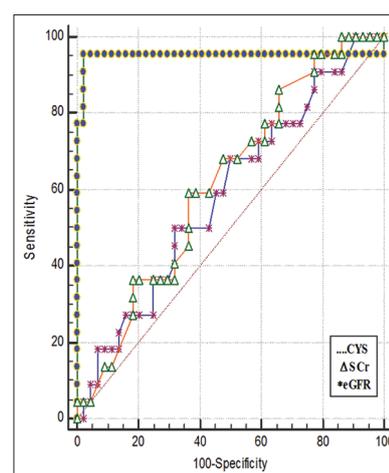


Fig. 6: Receiver operating characteristic, Cystatin C, serum creatinine, estimated glomerular filtration rate

responsiveness causing renal injury [22]. Studies conducted by Keller *et al.* observed that Cys C is related to inflammation and oxidative stress, the key pathogenic components of metabolic syndrome were elevated in diabetes with or without AKI. We observed serum Cys C significantly elevated in Group III compared to Group II and Group I.

This has clearly shown that the renal profile, the lipid profile, and oxidative stress parameters are grossly altered in Group III compared to Group II and Group I. However, these profiles are also altered in Group II compared to Group I but not to the extent the values observed in Group III.

Cys C correlated well with hs-CRP, NO, creatinine, ACR, and eGFR. Our study clearly showed that Cys C has superior correlation coefficients and greater ROC-plot AUC (0.867 to 0.989) values compared with SCR (0.489 to 0.734) and eGFR (0.460 to 0.708). Oxidative stress has been shown to induce the synthesis of Cys C mRNA and protein, reflecting a cellular defensive response to oxidative stress [23]. Several studies have demonstrated that measurement of plasma Cys C concentrations may be useful tool to diagnose AKI early but its superiority over SCR has not been universally demonstrated. A study done by Zhang *et al.* found that AUC of plasma Cys C to predict AKI was between 0.86 and 0.96. In contrast, urinary Cys C levels had only moderate diagnostic value, with a pooled AUC of 0.64 (95% confidence interval) [23]. Royackers *et al.* measured serial plasma and urinary Cys C prospectively in 151 Intensive Care Unit patients and assessed the performance for AKI prediction on day 1 and day 2 before the

RIFLE criteria for AKI were met. On day 2, plasma and urinary Cys C had an AUC for predicting AKI of 0.72 and 0.49, respectively. On day 1, the AUCs were 0.62 and 0.46, respectively. Plasma Cys C levels did not rise earlier than SCR [24].

Strengths of my study are that serum Cys C is a better marker compared to SCR, nitric oxide, and MDA levels were elevated in diabetes with AKI compared to healthy controls and diabetes without AKI. Limitations of our present study are small sample size and Group III subjects were not subclassified based on RIFLE criteria. Further studies are required with a combination of other advanced markers for early identification and detection of AKI.

In conclusion, the recognition that relatively trivial rises in creatinine may herald potentially disastrous sequela for T2DM patients has led to an increased awareness of potential AKI. Hopefully, the advent of new biomarkers will help defining the kidney at risk rather than relying simply on creatinine. In clinical practice for diagnosing acute myocardial infarction, an excellent biomarker such as high sensitive troponin I (AUC of 0.96) has been recently employed. It is important to acknowledge that it took years of measurement of troponin to become a routine clinical investigation in patients with cardiac chest pain.

To date, none of the new AKI biomarkers have undergone a similar rigorous assessment, but the current progress will hopefully lead to success and ultimately to improvement in patient outcome.

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