Serum Neutrophil Gelatinase-Associated Lipocalin and Cystatin C are early Biomarkers of Contrast-Induced Nephropathy After Coronary Angiography in Patients With Chronic Kidney Disease

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Serum Neutrophil Gelatinase-Associated Lipocalin and Cystatin C are early Biomarkers of Contrast-Induced Nephropathy After Coronary Angiography in Patients With Chronic Kidney Disease

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Abstract

We had previously reported on serum neutrophil gelatinase-associated lipocalin (NGAL) as an earlier biomarker of contrast-induced nephropathy (CIN) than serum creatinine (SCr) in 100 patients with chronic kidney disease undergoing coronary angiography.[1] We then compared serum NGAL to serum cystatin C (CysC) in the same group of patients. The SCr, estimated glomerular filtration rate, serum NGAL, and serum CysC were measured at baseline and various time points as appropriate postprocedure. The frequency of CIN was 11% (n = 11). Serum NGAL increased ≥25% from baseline at 24 hours in 7 patients with CIN ($P = .04$). Serum CysC increased ≥25% from baseline at 24 hours in 4 patients with CIN ($P = .008$). Changes in serum NGAL and serum CysC from baseline at 24 hours (Δ values) could diagnose CIN 24 hours earlier than SCr with serum NGAL showing a superior performance.

Keywords

neutrophil gelatinase-associated lipocalin, cystatin C, contrast-induced nephropathy, coronary angiography, chronic kidney disease

Introduction

The present study is a follow-up of the same patients in our initial report published in this journalon the usefulness of neutrophil gelatinase-associated lipocalin (NGAL) as an early marker of contrast-induced nephropathy (CIN) after coronary angiography.[1] The CIN is a recognized complication of cardiac catheterization. It has been reported to be the third leading cause of acute kidney injury (AKI) in hospitalized patients.[2] Patients with chronic kidney disease (CKD) are at increased risk of CIN. As serum creatinine (SCr) rises only after a few days following renal tubular injury,[3,4] it is insensitive for the early detection of CIN. Hence, earlier biomarkers of acute renal impairment postcoronary angiography are important to allow early intervention and prevention of progression of renal damage, especially in high-risk patients. Several biomarkers have been investigated for the early diagnosis of CIN.[5,6] These include serum NGAL and serum cystatin C (CysC).

The NGAL is a 25-kDa protein covalently bound to gelatinase from neutrophils, and both serum and urine levels are reported to rise as early as 2 to 4 hours postinsult, and it has been identified as a potential biomarker of AKI.[7,8] The CysC is an endogenous nonglycosylated 13-kDa protein which is produced at a relatively constant rate and released into the plasma by all nucleated cells in the body. It has a very low molecular weight and is readily filtered at the glomerulus. It is completely

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Table 1. Baseline Characteristics and Demographic Data of Patients.

Characteristics	Mean/Median
Age, years[a]	60.4 ± 8.3
Body mass index,[b] kg/m^2	26.6 (IQR ± 14.3)
Antiplatelet agent, (%)	96 (96)
ACEIs/ARBs, (%)	56 (56)
β-Blockers, (%)	77 (77)
Calcium antagonists, (%)	35 (35)
Statins, %	89 (89)
Hemoglobin,[a] 14.0-17.0 g/dL	12.71 ±1.53
Hematocrit,[b] 39.0-52.0%	38.1 (IQR ± 6.28)
Cholesterol,[b] <5.7 mmol/L	4.74 (IQR ± 1.47)
FBS,[b] 3.0-6.7 mmol/L	6.8 (IQR ± 3.57)
Serum creatinine,[a] 40-80 μmol/L	126.5 (IQR ±55)
eGFR,[b] mL/min/1.73 m^2	50.19 ± 16.16
Serum CysC, mg/L	1.39 (IQR ± 0.61)
Serum NGAL, ng/mL	102 (IQR ± 85)

Abbreviations: ACEI, angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers; eGFR, estimated glomerular filtration rate; FBS, fasting blood sugar; statins, hydroxymethylglutaryl coenzyme A reductase inhibitors; NGAL, neutrophil gelatinase-associated lipocalin; CysC, cystatin C; SD, standard deviation; IQR, interquartile range.
[a]Mean ± SD.
[b]Median ± IQR.

reabsorbed and catabolized and is not secreted by the tubular cells.[9,10] Due to these characteristics, serum CysC concentration is considered superior to SCr as a marker of AKI.[11] We therefore studied both serum NGAL and serum CysC as potential new biomarkers for the early detection of CIN among patients with CKD stages 2 to 4 (National Kidney Foundation [K/DOQI] classification) who underwent coronary angiography with/without angioplasty.

Patients and Methods

The present report is a follow-up of the same patients in our previous study of NGAL as an early marker of CIN after coronary angiography.[1] The CIN was defined as an increase in the baseline SCr ≥25% within 48 hours of exposure to contrast medium (CM) in the absence of an alternative etiology. This was a prospective observational study in which all patients with coronary artery disease electively admitted for coronary angiographic studies in our center, between October 2008 and November 2009. The patients were screened, and those with CKD stages 2 to 4 were recruited. Patients were excluded if they had AKI, acute myocardial infarction, end-stage renal disease, cardiogenic and/or septic shock, and exposure to nephrotoxic drugs or CM 48 hours prior to the study period.

All patients received intravenous normal saline at a rate of 1 mL/kg/h and oral N-acetylcysteine (NAC) 600 mg twice daily for 3 days 12 hours preprocedure. For SCr, blood was drawn from each patient at baseline prior to angiography and at 24 and 48 hours after the procedure. Blood samples for serum NGAL were taken pre-, at 4 hours, and24 hours postprocedure and were stored at −80°C until assay. Blood samples for serum CysC were collected pre- and at 24 hours postprocedure and were then stored at −80°C. Both serum NGAL and CysC levels were assayed after all patients were enrolled, the latter being delayed due to funding problem. The study protocol was approved by the Scientific Research and Ethics Board (Faculty of Medicine, Universiti Kebangsaan Malaysia), and all enrolled patients gave their informed consent.

Biochemical Analysis

For NGAL, all sera were initially diluted 20-fold using calibrator diluents and then measured with an enzyme-linked immunosorbent assay kit according to the manufacturer's protocol (R&D Systems, Minneapolis, Minnesota). A microplate spectrophotometer reader was used to measure the absorbance at 450 nm with the correction wavelength set at 540 nm. Serum CysC assays were performed using the particle-enhanced nephelometric immunoassay principle with CysC kits supplied by the Dade-Behring, Marburg, Germany. The SCr was assayed according to the standard routine laboratory methods (Jaffe colorimetric method). The estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease original equation.[12]

Statistical Analysis

Statistical analyses were performed using the SPSS Version 18 and MedCalc Version 11.5.1.0 (MedCalc Software, Mariakerke, Belgium). A $P < .05$ (2-tailed) was considered significant. Parametric data were expressed as mean ± standard deviation and nonparametric data as median ± interquartile range (IQR). As serum NGAL and serum CysC levels are not normally distributed, nonparametric tests were used to compare serum NGAL and serum CysC concentrations. Spearman correlation coefficients were used to assess the correlation between serum NGAL and serum CysC concentration with standard renal markers. Receiver–operating characteristic (ROC) curves and the area under the curve (AUC) were constructed to describe the performance of serum NGAL, serum CysC, and SCr measured as changes (Δ values) from the baseline to 24 hours post-CM exposure. The sensitivity, specificity, positive, and negative predictive values of these markers for predicting CIN were then calculated. The best cutoff values for biomarkers and SCr were chosen on the basis of maximum sensitivity and specificity.

Results

Baseline Characteristics and Demographic Data

A total of 100 consecutive patients with stable CKD stages 2 to 4 (KDOQI classification) were recruited. Their baseline demographic, clinical, and laboratory characteristics are as shown in Table 1. There were 79 males and 21 females with a mean age of 60.4 ± 8.3 years. Of these, 50% were Malays, 38% Chinese, and 2% Indians. Slightly more than half of the patients had never smoked (n = 52), 43 were past smokers, and 9 were still smoking. Major comorbidities included hypertension (n = 92), diabetes mellitus (DM; n =71), and dyslipidemia (n = 64).

Table 2. Changes in Serum Creatinine, eGFR, Serum CysC, and Serum NGAL Postcardiac Catheterization.

Variables	CIN (n = 11)	No CIN (n = 89)	*P*
Serum creatinine,[a] μmol/L			
At baseline	126 (87)	127 (54.5)	.08
24 hours after coronary angiography	119 (50.5)	131 (116)	.15
48 hours after coronary angiography	120 (51.5)	176 (110)	.006
eGFR,[b] mL/min/1.73 m^2			
At baseline	45.27 ± 53	50.79 ± 16.03	.28
24 hours after coronary angiography	39.27 ± 20.18	53.38 ± 16.35	.01
48 hours after coronary angiography	30.63 ± 14.15	51.85 ± 16.31	≤.001
Serum CysC,[b] mg/L			
At baseline	1.51 (1.03)	1.27 (0.57)	.11
24 hours after coronary angiography	2.03 (1.39)	1.28 (0.66)	.008
Serum NGAL,[b] ng/mL			
At baseline	89.8 (116.8)	102.4 (79.4)	.90
4 hours after coronary angiography	110.4 (115.4)	96.6 (71.3)	.32
24 hours after coronary angiography	138.6 (766)	99.4 (79.9)	.04

Abbreviations: eGFR, estimated glomerular filtration rate; CysC, cystatin C; NGAL, neutrophil gelatinase-associated lipocalin; CIN, contrast-induced nephropathy; SD, standard deviation.
[a]Median ± interquartile range.
[b]Mean ± SD.

Angiographic procedures performed included coronary angiography only (n = 77), coronary angiography with added angioplasty (n = 16), and angioplasty only at the current session (n = 7). The median duration of the procedure was 30 minutes (IQR 31.5), and the median volume of CM administered 80 mL (IQR 30). Iso-osmolar CM (''Iodixanol'') was used in the majority of the patients (n = 68), and low osmolar CM (''Iohexol'') was used in 32 patients.

Frequency of CIN

The frequency of CIN was 11% (11 of 100), and 1 patient required dialysis.

Serum Creatinine, eGFR, Serum CysC, and Serum NGAL Measurements

At baseline, there were no differences in the serum levels of SCr ($P = .08$), eGFR ($P = .28$), CysC ($P = .11$), and NGAL ($P = .90$) between patients who developed CIN and those who did not. These changes are summarized in Table 2.

The SCr in the CIN group started to rise at 24 hours postprocedure ($P = .15$) but achieved significance only at 48 hours ($P = .006$). There were marked declines in the eGFR in the CIN group at 24 and 48 hours postprocedure ($P = .01$, $P \leq .001$, respectively). At 24 hours, serum CysC increased ≥25% from baseline in 4 patients with CIN (4 of 11; $P = .008$) but did not change in the remaining 7 (7 of 11). At 4 hours, serum NGAL was not significantly different from that at baseline between those who developed CIN and those who did not ($P = .32$). However at 24 hours, serum NGAL increased ≥25% from the baseline in 7 patients with CIN (7 of 11; $P = .04$) but did not change in the other 4 (4 of 11). Serum NGAL also increased by ≥25% in 12 (12 of 89) non-CIN patients (Fischer exact test = 0.001).

In general, those with a stable SCr at 48 hours post-CM exposure were discharged later in the day per protocol. One patient developed CIN only after 72 hours and was kept in the hospital. This patient was initially placed in the non-CIN group (based on SCr) at 48 hours but in retrospect had a rise in both serum CysC and serum NGAL ≥25% at 24 hours.

Correlations of Serum CysC and Serum NGAL With Standard Renal Markers

At baseline and at 24 hours post-CM exposure, both serum NGAL and serum CysC were highly associated. Both were also significantly associated with SCr and eGFR at both time points (Figures 1-3).

Characteristics of Serum CysC and Serum NGAL for the Early Diagnosis of CIN

The results of the ROC analyses for the above parameters are displayed in Table 3. The AUC of the changes in SCr between baseline and 24 hours (Δ values) was 0.747 (95% confidence interval [CI]: 0.65-0.82, $P = .008$) and the change in serum CysC pre- and post-CM exposure at 24 hours (Δ values) was 0.80 (95% CI: 0.70-0.87, $P = .001$);whereas in serum NGAL, the AUC change at these same time points (Δ values) was 0.845 (95% CI: 0.75-0.91, $P < .001$), which was higher than that of serum CysC. In addition, the AUCs of both the biomarkers were superior to that of SCr (Figure 4).

At 24 hours after CM exposure, changes in serum NGAL with concentrations >17.7 ng/mL had a sensitivity of 72.7% and a specificity of 76.4% for the early detection of CIN. In comparison, serum CysC with concentration changes >0.19 mg/L had lower sensitivity (63.64%) but higher specificity (88.76%) at similar time points.

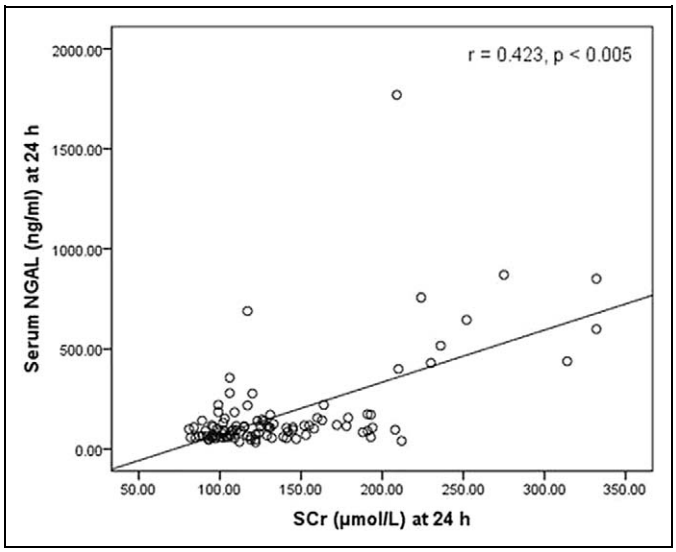

Figure 1. Serum neutrophil gelatinase-associated lipocalin (NGAL) and serum creatinine (SCr) 24 hours postcoronary angiography.

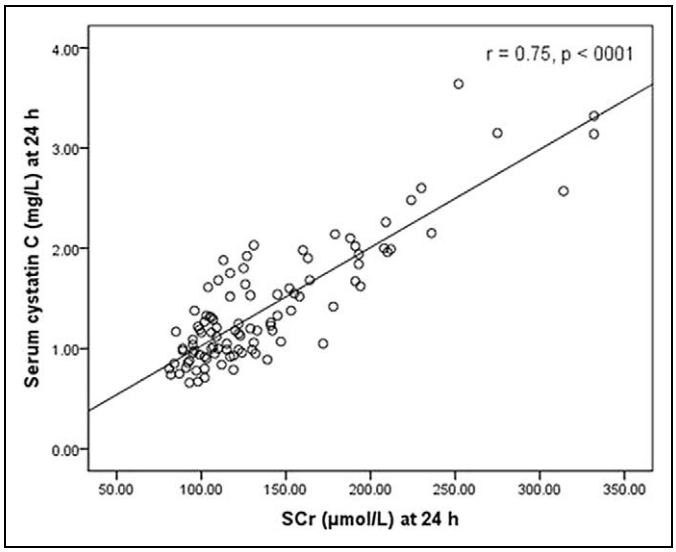

Figure 2. Serum cystatin C (CysC) and serum creatinine (SCr) 24 hours postcoronary angiography.

Discussion

The conventional definition of CIN in patients undergoing radiocontrast studies is a $\geq$25% rise in the baseline SCr.[13] Patients with CKD are at a higher risk of cardiovascular disease (CVD) and vice versa.[14] Several risk scores have been developed to identify the patients at higher risk of CIN prior to CM administration. The Global Registry of Acute Coronary Events (GRACE) score was developed by Raposeiras-Roubín et al for use in patients with acute coronary syndrome and normal renal function.[15] Patients with a GRACE score >140 are at increased risk of developing CIN.[15] Fu et al established a risk score named CR4EATME3AD3 to assess the risk of CIN in elderly patients undergoing percutaneous coronary intervention

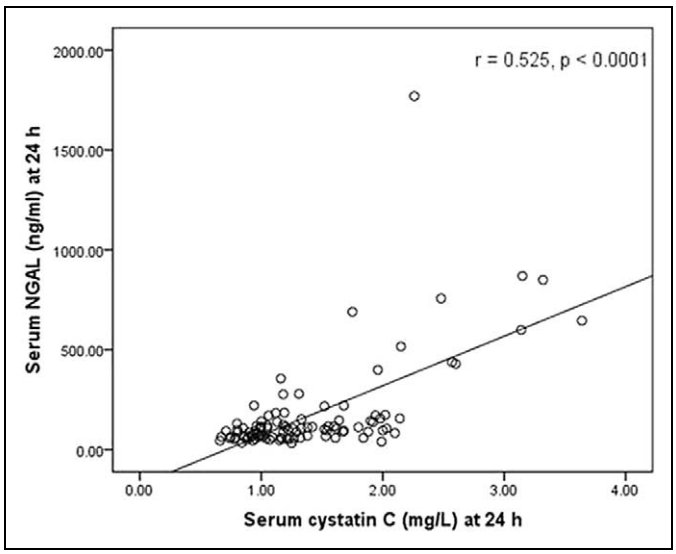

Figure 3. Serum neutrophil gelatinase-associated lipocalin (NGAL) and serum cystatin C (CysC) 24 hours postcoronary angiography.

(PCI).[16] This score was derived by extracting 1 character from the following 9 risk factors: contrast volume > 200 mL, eGFR < 60 mL/min/1.73 m^2, emergency PCI, age > 70 years, hypotension, history of myocardial infarction (MI), left ventricular ejection fraction < 45%, anemia, and DM. The patients were stratified according to the total score as follows: low-risk group (score $\leq$4), medium-risk group (scores 5-8), high-risk group (scores 9-12), and very high-risk group (score $\geq$13). This score was then validated in another 277 elderly patients and found to be a good predictor of CIN.[16]

Coronary angiography with or without angioplasty is increasingly performed with a 2- to 3-day turnaround hospital stay. Hence, early biomarkers of CIN other than SCr would greatly improve the patient's safety, especially in this high-risk CKD subpopulation. Indeed, there is scant data on CIN in patients with CKD in the literature.

We had earlier reported on the use of NGAL as an early marker of CIN after coronary angiography in this journal.[1] This article further explored the utility of CysC when compared with serum NGAL, SCr, and eGFR as biomarkers of CIN in patients with CKD stages 2 to 4 in our local setting. We first performed an exhaustive review of the previously published studies on the use of these 2 biomarkers in the diagnosis of AKI, especially among patients undergoing elective cardiac radioangiographic studies or cardiopulmonary bypass surgery to identify the optimal times for the rise in these 2 markers post-CM exposure. The serum levels of both these markers have been demonstrated to precede any SCr rise at 24 hours attesting their usefulness as early indicators of AKI. The protocol was then designed so as to maximize anticipated results for a successful outcome within the constraints of our budget. Hence, blood samples for the assay of serum NGAL were taken at baseline, 4 hours, and 24 hours postprocedure, and those for serum CysC were taken at baseline and at 24 hours post-CM exposure. Ideally, the serum levels of both these markers at 48 hours

Table 3. Comparison of biomarker test after coronary angiography for early detection of CIN

Value	AUC (95% CI)	p value	Cut off value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Serum creatinine, μmol/L Δ1: 24 h-0	0.747 (0.65 - 0.82)	0.008	15	54.5 (23.4 - 83.3)	78.6 (68.7 - 86.6)	23 (9.1- 45.6)	93.3 (85.1 - 97.8)
Serum CysC, mg/L Δ1: 24 h-0	0.800 (0.70 - 0.87)	0.001	0.19	63.6 (30.8 - 89.1)	88.7 (80.3 - 94.5)	32 (13.3-59.0)	95.2 (87.3 - 99.1)
Serum NGAL, ng/ml Δ1: 24 h-0	0.845 (0.75 - 0.91)	<0.001	17.7	72.7 (39.0 - 94.0)	76.4 (66.2 - 84.8)	33 (14.7 - 54.2)	95.8 (88.1 - 99.1)

Abbreviations: AUC, area under curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; CM, contrast medium; CysC, cystatin C; NGAL, neutrophil gelatinase-associated-lipocalin.

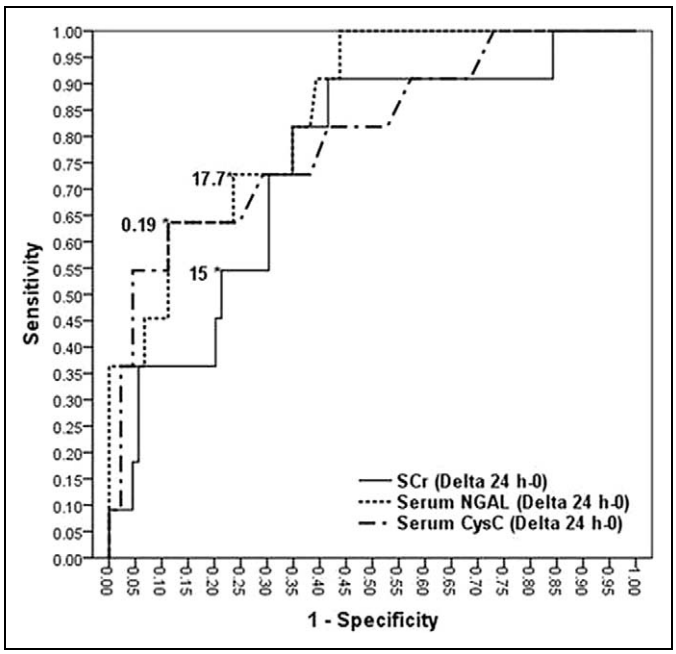

Figure 4. Receiver–operating characteristic curves showing the performance characteristics of changes at 24 hours from baseline (Δ values) in serum creatinine (SCr), serum neutrophil gelatinase-associated lipocalin (NGAL) and serum cystatin C (CysC) for early diagnosis of contrast induced nephropathy. The best cutoff Δ values at 24 hours from baseline for predicting CIN was 15 μmol/L for SCr (sensitivity 54.5%, specificity 78.6%), 0.19 mg/L for serum CysC (sensitivity 63.6%, specificity 88.7%), and 17.7 ng/mL for serum NGAL (sensitivity 72.7%, specificity 76.4%).

postprocedure should have also been ascertained, but our budget precluded this. These assay times are also more representative of standard blood collection in routine clinical practice.

The use of NAC as prophylaxis against CIN has been extensively evaluated.[17-22] However, its efficacy remains controversial. Several meta-analyses have reported that NAC has beneficial effect in preventing CIN,[17-19] but on others have shown the contrary.[20-22] Many other pharmacological agents have also been investigated[23-27] and include furosemide,[24,27] dopamine,[28] fenoldopam,[28,29] calcium channel blockers,[28,30] high doses of atorvastatin,[31,32] and even ascorbic acid[33] (latter as antioxidant). However, their roles remain uncertain.

Despite the routine prophylactic measures of administration of oral NAC and saline hydration, the frequency of CIN in our CKD cohort was 11%. The reported incidences of CIN in patients who underwent coronary radioangiographic studies and received similar prophylactic measures but have essentially normal SCr ranged between 4% and 8%.[34] This may be explained by the underlying CKD of our study population, as CKD predisposes to AKI especially in older patients with coronary artery disease and multiple comorbidities, which reflect their atherosclerosis burden.[13]

In our patients, the SCr reached a peak only at 48 hours in those who developed CIN. This concurs with that reported by others.[35,36] All these studies including ours show that SCr is a late marker of AKI.[37] Bachorzewska-Gajewska et al demonstrated that serum NGAL levels increased significantly at 2, 4, and 8 hours after PCI, while the rise in urinary NGAL levels occurred later at 4, 8, and 24 hours postprocedure, but SCr remained unchanged.[5] In another study, Bachorzewska-Gajewska et al also reported that both serum and urinary NGAL rose significantly after 2 to 4 hours and 4 to 8 hours, respectively, in those patients who developed CIN after elective cardiac catheterization.[38] Similarly, Shaker et al, in a smaller prospective study of 30 patients with normal SCr undergoing coronary angiography, found that serum NGAL increased significantly at 4 hours postprocedure and lasted for 24 hours.[39]

Our study showed quite the converse—the serum NGAL at 4 hours did not change in all our study patients. This may be due to the fact that all had received the standard protocol of hydration with IV normal saline and prophylactic oral NAC, 12 hours pre-exposure to CM. In our study, at 24 hours serum NGAL increased ≥25% from baseline to 24 hours in the majority of the patients with CIN (7 of 11 = 64%), while serum CysC at 24 hours increased >25% from baseline only in 4 patients with CIN (4 of 11 = 36%). Serum NGAL also increased in 12 (12 of 89 = 13.4%) of the non-CIN patients. One of these also had a raised serum CysC and was subsequently diagnosed to have CIN and was kept in the hospital. The rest were discharged 48 hours post-coronary studies per our current practice. We surmise that these patients could have had "incipient CIN" at the time of discharge. As well, they may have had missed or subclinical CIN postdischarge but recovered spontaneously, since none were readmitted during the study. This remains a conjecture.

This study provides further evidence to those of the few earlier reports that, in patients undergoing coronary angiographic studies, serum NGAL and serum CysC were early biomarkers

of CIN compared with SCr.[5,6,38,39] These markers facilitate the diagnosis of CIN at least 24 hours earlier than that of SCr. In the present study, both SCr and eGFR were highly correlated with serum NGAL and serum CysC at baseline and at 24 hours after coronary intervention. These findings are in agreement with those of the other reports.[5,39]

In a prospective observational study of 311 patients with mild or moderate CKD undergoing elective PCI, Liu et al found that the plasma NGAL rose at 2 hours and peaked at 4 hours postprocedure, whereas plasma CysC increased at 2 hours and peaked only at 24 hours. Increase in NGAL $\geq 25\%$ at 4 hours after CM exposure had higher diagnostic values of CIN.[40] Briguori et al performed a large prospective study of 410 patients with CKD undergoing coronary catheterization, in which all patients received prophylactic IV sodium bicarbonate and NAC.[41] They reported that an increase in serum CysC $\geq 10\%$ at 24 hours post-CM exposure was a reliable marker for the early diagnosis and prognosis of CIN.[41] Similarly, Rickli et al observed an early rise in serum CysC at 24 hours post-procedure compared with SCr in their study cohort ($n = 41$).[6] Despite "adequate hydration" in a similar patient cohort, Shaker et al noted that serum CysC also rose significantly at 24 hours postprocedure.[39] In contrast, Bachorzewska-Gajewska et al noted that serum CysC increased significantly at 8 and 24 hours after PCI despite pretreatment with oral hydration only.[5] However, Ishibashi et al demonstrated that, in their study patients the baseline serum CysC was significantly higher in those with moderate renal insufficiency who had CIN. However, the prophylactic measures used were not declared.[42] They concluded that baseline serum CysC was an early predictor of CIN.[42]

Ling et al had shown that in addition to urinary NGAL, urinary IL-18 was also significantly increased at 24 hours after coronary angiography compared with SCr.[43] The prophylactic measures were not stated in their study.[43]

Analysis of the ROC curves of the changes in serum NGAL and serum CysC at 24 hours postprocedure from baseline values demonstrated that either can be used for the early diagnosis of CIN. However, serum NGAL had higher sensitivity and specificity compared to serum CysC. Both were superior compared to the conventional wait for SCr to rise further at 48 to 72 hours postprocedure. With the use of these 2 early biomarkers of AKI, affected patients can then be kept in hospital, while the rest can be safely discharged home without major concern for the subsequent development of CIN, especially in this high-risk CKD population.

Albeit a small study, this is nonetheless larger than those previously reported on the usefulness of early biomarkers of CIN/AKI in this high-risk group of cardiac patients with CKD. Its strength lies in the fact that it was prospective, the patient population was homogenous for CKD, and the calculated sample size required to demonstrate significance with a power of 80% was achieved. In addition, all patients received the current standard prophylactic protocol with IV saline hydration and oral NAC.

In conclusion, this study demonstrated that both serum NGAL and serum CysC are superior biomarkers than SCr and eGFR for the early diagnosis of CIN in patients with CKD undergoing elective cardiac angiographic studies. In particular, serum NGAL at 24 hours appears to be a superior predictor of risk of CIN.

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Author's Note

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Declaration of Conflicting Interests

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