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# Significance of Cystatin C for Early Diagnosis of Contrast-Induced Nephropathy in Patients Undergoing Coronary Angiography

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**Background:** Contrast-induced nephropathy is acute kidney injury caused by contrast medium exposure. Serum creatinine is the clinical diagnostic standard, but it does not yield quick results. The serum level of cystatin C is stable and it can reflect renal function sensitively. The study aimed to assess the usefulness of cystatin C for early diagnosis of contrast-induced nephropathy in patients undergoing coronary angiography.





**Material/Methods:** We included 300 patients who underwent CAG. According to the sCr at 48 h, patients were divided into 2 groups: CIN group and non-CIN group. Their demographics and basal renal function were recorded. Changes in sCr, Cys C, and e GFR were compared at the same time. ROC analysis was used to assess the sensitivity and specificity of Cys C in the early diagnosis of CIN.

**Results:** Comparison of basal renal function and serum level of Cys C showed no significant differences between the 2 groups. Serum level of Cys C increased significantly at 24 h ( $p < 0.001$ ), and sCr increased significantly at 48 h. ROC analysis showed that the AUC of the change in Cys C between baseline and 24 h was 0.936 (95% CI: 0.879–0.992,  $p = 0.000$ ) and the optimum cut-off level was 0.26 mg/L (sensitivity=89.7% and specificity=95.6%).

**Conclusions:** The concentration change of Cys C is better than sCr as a biomarker in the early detection of CIN.

**MeSH Keywords:** **Acute Kidney Injury • Creatinine • Cystatin C**

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## Background

With the development of enhanced radiation technology and interventional techniques, effective diagnosis and treatment are possible for patients with coronary and other peripheral vascular diseases. However, therapeutic effects always come with complications, including vascular perforation, dissection, and contrast-induced nephropathy (CIN). Among these complications, CIN has been demonstrated to be associated with irreversible renal insufficiency, prolonged hospitalization, and high cost. Additionally, CIN has unfavorable early and late prognosis [1]. Therefore, it is important to diagnose CIN and provide treatment as soon as possible, which depends on the early diagnosis. Contrast-induced nephropathy, also referred to as contrast-induced acute kidney injury, has become the third leading cause of hospital-acquired acute kidney injury. Half of these cases are caused by repeated exposure to contrast media during cardiac catheterization and coronary angiography (CAG) [2]. Contrast-induced nephropathy is defined as an increase in serum creatinine (sCr) concentration  $>0.5$  mg/dL or a minimum of 25% increase from baseline within 72 h after contrast administration, without evidence of other causes [3,4]. Detection of serum creatinine is the foundation for the diagnosis of CIN; however, the sCr level may not be determined quickly enough to reflect decreased renal function, and sCr level is affected by factors such as age, sex, and muscle mass [5]. In conclusion, sCr is not a perfect glomerular filtration rate (GFR) biomarker and is not sensitive enough to detect early changes in GFR. Thus, there is great need for a more sensitive and reliable biomarker for predicting the occurrence of acute kidney injury (AKI) or CIN.

Numerous clinical trials have been sought to find discover an appropriate biomarker for early and precise assessment of renal function [6–8]. Disappointingly, many biomarkers have been confirmed to be useless, including neutrophil gelatinase-associated lipocalin, urinary liver type fatty acid-binding protein (L-FABP), and urinary kidney injury molecule 1 [9,10]. Serum Cystatin C (Cys C) is a non-glycosylated protein with low molecular mass (13 kDa). It is a cysteine protease inhibitor synthesized by all nucleated cells and released into the blood by glomerular filtration [11]. Most importantly, external factors have little effect on the serum level of Cys C. Previous studies have demonstrated its utility in detection of diabetic mellitus and hypertensive nephropathy [12].

In the present study we sought to determine the ability of Cys C to predict the occurrence of CIN in patients who underwent CAG and intervention, and we compared the sensitivity and specificity of Cystatin C and serum creatinine in the diagnosis of CIN.

## Material and Methods

### Study population

From January 2015 to May 2015, a total of 300 consecutive patients who underwent only diagnostic coronary angiography for suspected coronary heart disease were enrolled in the study. The study was done at Department of Cardiology, West China Hospital. It was funded by the National Natural Science Foundation of China (No. 81200153), the Science Foundation of Health Department, Sichuan Province (No. 20120213), the Science Foundation of Science and Technology Department, Sichuan Province (No. 2015SZ0180), and the Science Foundation of the Science and Technology Department, Sichuan Province (No. 2014JY0204). Our study was approved by the institutional ethics committee. Each enrolled patient signed an informed consent form for the use of related data. The exclusion criteria included: 1) patients with chronic nephropathy (CKD stage 2 to 4); 2) patients with other contrast exposure within 1 week, or exposure to nephrotoxic drugs; 3) patients with previous kidney transplantation, cardiac dysfunction, thyroid disorder, or cancer; 4) patients who were unable to understand the study content or provide consent; 5) patients younger than 18 or older than 80 years old; and 6) patients simultaneously participating in other studies.

### Study design

Patient data were recorded for feasibility analysis at admission, including sex, age, body mass index (BMI), left ventricular ejection fraction (LVEF), hypertension, and diabetes mellitus, as well as use of oral medicines, especially angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEI/ARB), and statins. Fasting baseline renal function and serum level of Cys C were measured as basal indicators 24 h before CAG. As another important evaluation indicator, estimated GFR (eGFR) was calculated by the Levey modification of the Modification of Diet in Renal Disease (MDRD) formula [13]. Coronary angiography was performed according to current clinical guidelines, including preventive hydration. A standard dose (3–5 ml/kg) of iodinated contrast medium (iohexol, GE Pharmaceutical, Shanghai) was used. In the process of angiography, we recorded the operation time and the infusion dose of contrast medium. Serum samples were collected at 12, 24, and 48 h after CAG for measuring postoperative levels of Cys C and creatinine. All blood samples were analyzed by the clinical laboratory in our hospital.

According to the definition of CIN, we calculated the change in creatinine and confirmed the occurrence of CIN. Then, all the patients were assigned into 2 groups based on whether CIN occurred. After grouping, we compared the collected demographics, medication usage, exposure to contrast

**Table 1.** Baseline characteristics.

Characteristic	CIN (n=29)	Non-CIN (n=271)	P-value
Age (years)	65.63±10.43	62.18±8.96	0.048*
Female (% , n)	69.0 (20)	37.3 (101)	0.037*
BMI (kg/m <sup>2</sup> )	23.45±2.79	22.97±2.56	0.191
Hypertension (% , n)	48.3 (14)	47.2 (128)	0.536
Diabetes mellitus (% , n)	34.5 (10)	35.8 (97)	0.547
Tobacco use (% , n)	31.0 (9)	37.3 (101)	0.404
LVEF (≤50%)	13.8 (4)	12.9 (35)	0.543
Medication history			
ACEI/ARB (% , n)	37.9 (11)	31.4 (85)	0.368
β-blocker (% , n)	17.2 (5)	18.8 (51)	0.548
CCB (% , n)	20.7 (6)	18.5 (50)	0.483
Statins (% , n)	62.1 (18)	74.2 (201)	0.342
Baseline renal function			
sCr (μmol/L)	77.17±13.72	80.84±10.45	0.086
eGFR (mL/min/1.73 m <sup>2</sup> )	92.91±5.67	91.29±6.54	0.079
Cys C (mg/L)	1.28±0.23	1.23±0.19	0.133
Infusion dose of CM (mL)	101.85±11.38	97.86±10.94	0.040*
Operation time (min)	40.34±5.87	42.06±4.99	0.069

BMI – body mass index; LVEF – left ventricular ejection fraction; ACEI – angiotensin-converting enzyme inhibitors; ARB – angiotensin receptor blocker; CCB – calcium channel blockers; sCr – serum creatinine; eGFR – estimated glomerular filtration rate; Cys C – cystatin C; CM – contrast medium.

medium, and serum levels of Cys C and creatinine at different time points.

### Statistical analysis

Results are expressed as mean ± standard deviation (SD) for continuous variables and frequencies for categorical variables. Differences between groups were examined by nonparametric test and chi-square test for continuous and categorical variables, respectively. Other variables that were significantly associated with outcome were fed into the model in a stepwise procedure. An alpha value of 0.05, corresponding to a p value <0.05, served as the criterion for establishing statistical significance. The diagnostic sensitivity and specificity for the early diagnosis of CIN based on the serum level of Cys C and sCr at 24 h were calculated by use of ROC curve analysis and AUC assessment. Analysis was performed using SPSS for Windows (SPSS Inc., Version 19.0, Chicago, IL) and STATA (Version 16.0).

## Results

### Baseline characteristics

A total of 300 patients (179 males, 121 females) with a mean age of 63.47±9.92 were included in the final study. According to the definition of CIN, patients with a >25% relative increase of serum creatinine, or an increase in concentration of serum creatinine of at least by 44 mmol/L from the baseline 48 h after CAG, were included in the CIN group. Twenty-nine patients developed CIN and none of them developed acute kidney failure. The other 271 patients were included in the non-CIN group. We analyzed and compared the demographics of the 2 groups. As is shown in Table 1, patients in the CIN group were significantly older than those in the non-CIN group (p=0.048), and the CIN group had a higher proportion of females than the non-CIN group (p=0.037). However, the differences in the ratio of hypertension, diabetes mellitus, smoking, and cardiac dysfunction were not statistically significant. Medication history was also recorded to analyze the effect on renal function, though there were no obvious distinctions between the 2 groups (all p>0.05). Previous researchers

**Table 2.** Variation tendency of creatinine and Cystatin C.

	CIN (n=29)	Non-CIN (n=271)	P-value
Serum creatinine (μmol/L)			
Baseline	77.17±13.72	80.84±10.45	0.086
12 hours after CAG	77.89±12.56	79.97±11.12	0.199
24 hours after CAG	84.88±11.89	82.74±13.47	0.185
48 hours after CAG	100.23±10.32***	81.89±12.76	<0.001***
p-value	<0.001***	0.085	
Serum cystatin C (mg/L)			
Baseline	1.28±0.23	1.23±0.19	0.133
12 hours after CAG	1.39±0.42	1.32±0.24	0.192
24 hours after CAG	1.81±0.33***	1.35±0.22	<0.001***
48 hours after CAG	1.64±0.52**	1.22±0.17	0.001***
p-value	<0.001***	0.121	
eGFR (mL/min/1.73 m <sup>2</sup> )			
Baseline	92.91±5.67	91.29±6.54	0.079
12 hours after CAG	90.25±6.01	91.01±5.98	0.261
24 hours after CAG	85.62±6.23	89.93±6.34	0.001***
48 hours after CAG	71.89±5.52***	90.85±6.61	<0.001***
p-value	<0.001***	0.285	

CAG – coronary angiography; eGFR – estimated glomerular filtration rate.

have used infusion dose of contrast medium and the exposure time as important indicators of CIN, and we demonstrated the importance of these 2 indicators in our study. Patients in the CIN group received significantly larger doses of contrast medium ( $p=0.040$ ) and the operation time was longer compared to the non-CIN group. These significant differences revealed the cause of CIN. Comparison of basal renal function and serum level of Cys C showed no significant differences between the 2 groups.

### Changes in sCr and Cys C

As an important procedure in our study, we measured the serum levels of creatinine and cystatin C at various time points. Compared vertically, the rise in serum Cys C was the maximum at 24 h, which was significantly higher than baseline ( $p<0.001$ ). Then, the level declined, but still remained higher at 48 h ( $p<0.01$ ). The serum level of creatinine in the CIN group peaked at 48 h ( $p<0.001$ ) and was not significantly different at 24 h. eGFR had decreased at 24 h and had even more obviously decreased at 48 h ( $p<0.001$ ). It is obvious that the change in serum level of Cys C occurred prior to the change in serum level of creatinine. In the non-CIN group, the differences in sCr

and Cys C before and after the procedure were both non-significant. Compared horizontally, the difference in Cys C level between the 2 groups became apparent at 24 h ( $p<0.001$ ) and lasted until 48 h. The serum level of sCr in the CIN group was significantly higher than that in the non-CIN group only at 48 h, and the difference was not obvious at 24 h. Moreover, compared with non-CIN group, eGFR decreased from 24 h to 48 h in the CIN group, and both differences were significant ( $p<0.001$ ). Table 2 shows that the change in serum level of Cys C became significantly different before that of sCr.

### Significance of Serum Cys C and sCr for the Early Diagnosis of CIN

To study the significance of Cys C and sCr in CIN, we chose the serum levels at different time points for ROC analysis. The area under the curve (AUC) of Cys C at 12 h and 24 h was 0.735 (95% CI: 0.648-0.823,  $p=0.000$ ) and 0.928 (95% CI: 0.870–0.987,  $p=0.000$ ), respectively, and the optimum cut-off level was 1.315 mg/L (sensitivity=79.3% and specificity=55.4%) and 1.55 mg/L (sensitivity=82.8% and specificity=97.8%), respectively. The AUC of changes in Cys C between baseline and 12 h and 24 h were 0.758 (95% CI: 0.668–0.847,

**Table 3.** ROC analysis.

	AUC	95% CI	P value	Cut-off	Sensitivity (%)	Specificity (%)
Cys C (12 h)	0.735	0.648–0.823	0.000	1.315 mg/L	79.3	55.4
Cys C (24 h)	0.928	0.870–0.987	0.000	1.55 mg/L	82.8	97.8
Cys C ( $\Delta$ 12 h)	0.758	0.668–0.847	0.001	0.185 mg/L	79.3	95.3
Cys C ( $\Delta$ 24 h)	0.936	0.879–0.992	0.000	0.26 mg/L	89.7	95.6
Cys C ( $\Delta$ 24 h%)	0.923	0.875–0.972	0.000	17.15%	79.3	87.8
sCr (24 h)	0.733	0.640–0.825	<0.001	73.55 $\mu$ mol/L	89.7	43.5

Cys C – cystatin C; sCr – serum creatinine;  $\Delta$ 12 h – serum level changes between 12 hours and baseline;  $\Delta$ 24 h – serum level changes between 24 hours and baseline.

$p=0.001$ ) and 0.936 (95% CI: 0.879-0.992,  $p=0.000$ ), respectively, and the optimum cut-off level was 0.185 mg/L (sensitivity=79.3% and specificity=95.3%) and 0.26 mg/L (sensitivity=89.7% and specificity=95.6%), respectively. We calculated the increased percentage of Cys C between 24 h and baseline. The AUC was 0.923 (95% CI: 0.875–0.972,  $p=0.000$ ) and the optimum cut-off level was 17.15% (sensitivity=79.3% and specificity=87.8%). When we chose the serum level of creatinine at 24 h for ROC analysis, the AUC was 0.733 (95% CI: 0.640–0.825,  $p<0.001$ ) and the optimum cut-off level was 73.55  $\mu$ mol/L (sensitivity=89.7% and specificity=43.5%). According to the results in Table 3, although the AUCs of Cys C at 24 h and Cys C changes at 24 h were similar, serum Cys C with concentration changes  $>0.26$  mg/L had higher sensitivity (89.7% vs. 82.8%) but lower specificity (95.6% vs. 97.8%) for differentiating CIN and non-CIN.

## Discussion

Contrast-induced nephropathy is a well-known complication that can be observed after some enhanced radiologic and angiographic examinations and treatments. The injury is generally mild and transient but can result in lasting renal dysfunction and it is associated with increased morbidity and mortality [14]. However, the mechanism responsible for CIN remains unknown. Several probable pathways are thought to be associated with the mechanism of CIN. One is the influence of renal hemodynamics caused by contrast medium exposure and the other is the direct cytotoxicity of contrast medium to renal tubular epithelial cells [15]. In addition, there are no effective therapies for the acute kidney injury caused by contrast medium, so the only option is to prevent it from happening [16,17]. Early detection and early diagnosis have been the priority in CIN. Nowadays, serum level of creatinine is widely used and recognized as a diagnostic clinical indicator of CIN. However, sCr could be affected by numerous factors like age, sex, and muscle mass. Because of the reserve

capacity of the kidneys, concentrations of sCr can be within the reference range with a certain degree of impaired renal function. In our study, the level of sCr increased significantly 48 h after exposure and was obviously higher than in the non-CIN group ( $p<0.001$ ). Therefore, sCr is not efficient and accurate enough as a diagnostic indicator of CIN. These limitations prompted us to look for another indicator that can reflect damaged renal function in early stages.

We chose Cys C for the detection of CIN in our study. The results showed that the serum level of Cys C significantly increased at 24 h in CIN patients, which was before the increase in sCr. The variation tendency of Cys C in the non-CIN group was not obvious, as opposed to the increase in the CIN group ( $p=0.121$  vs.  $p<0.001$ ). ROC analysis further demonstrated the significance of Cys C in early diagnosis of CIN. Cys C is a 13-kDa non-glycosylated protein that is a cysteine protease inhibitor. It is produced by almost all nucleated cells at a constant rate and removed from the blood by glomerular filtration. The kidneys are the only organs that clear Cys C, and the serum level of Cys C only depends on GFR. Therefore, in theory, the concentration change of Cys C can reflect the change of GFR more sensitively than sCr [18]. Previous researches provided similar evidence of the superiority of Cys C [19]. Bachorzewska-Gajewska et al. found the same variation tendency of Cys C after contrast medium exposure [20]. Their study including 410 patients with chronic kidney disease indicated that Cys C is a reliable biomarker and predictor for the early diagnosis of CIN [18]. However, the form of Cys C used for CIN diagnosis is still in dispute.

Our study again verified the significant predictors of CIN, including age, female sex, the dose of contrast medium, and the exposure time. Most importantly, we revealed the potential association between the increase of Cys C and the occurrence of CIN. A certain increase of the concentration of Cys C is sensitive and specific for the prediction of CIN after contrast medium exposure. Despite of the achievements of our study,



it had certain deficiencies. Firstly, we only included patients with normal renal function at baseline, which limited the observations of the significance of Cys C in patients with chronic kidney disease. Then, we excluded patients who underwent PCI from our study, leading to insufficient contrast medium exposure. In addition, we analyzed the different forms of Cys C for the early diagnosis of CIN and results showed similar significance among these indicators. Therefore, the best form of Cys C for the diagnosis of CIN still remains uncertain. Further studies on early diagnosis of CIN are needed.

## References:

1. McCullough PA: Contrast-induced acute kidney injury. *J Am Coll Cardiol*, 2008; 51: 1419–28
2. McCullough P. Outcomes of contrast-induced nephropathy: Experience in patients undergoing cardiovascular intervention. *Catheter Cardiovasc Interv*, 2006; 67: 335–43
3. Kelly AM, Dwamena B, Cronin P et al: Meta-analysis: Effectiveness of drugs for preventing contrast-induced nephropathy. *Ann Intern Med*, 2008; 148: 284–94
4. Maliborski A, Zukowski P, Nowicki G, Boguslawska R: Contrast-induced nephropathy – a review of current literature and guidelines. *Med Sci Monit*, 2011; 17(7): RA199–204
5. Bellomo R, Kellum JA, Ronco C: Defining acute renal failure: Physiological principles. *Intensive Care Med*, 2004; 30: 33–37
6. Hirschberg R, Kopple J, Lipsett P et al: Multicenter clinical trial of recombinant human insulin-like growth factor I in patients with acute renal failure. *Kidney Int*, 1999; 55: 2423–29
7. Devarajan P. Biomarkers for the early detection of acute kidney injury. *Curr Opin Pediatr*, 2011; 23: 194–200
8. Ugur M, Uluganyan M, Ekmekci A et al: Combination of hemoglobin and left ventricular ejection fraction as a new predictor of contrast induced nephropathy in patients with non-ST elevation myocardial infarction. *Med Sci Monit*, 2014; 20: 967–73
9. Kato K, Sato N, Yamamoto T et al: Valuable markers for contrast-induced nephropathy in patients undergoing cardiac catheterization. *Circ J*, 2008; 72: 1499–505
10. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E et al: NGAL (neutrophil gelatinase-associated lipocalin) and cystatin C: Are they good predictors of contrast nephropathy after percutaneous coronary interventions in patients with stable angina and normal serum creatinine? *Int J Cardiol*, 2008; 127: 290–91
11. Shlipak MG, Ix JH, Bibbins-Domingo K et al: Biomarkers to predict recurrent cardiovascular disease: the Heart and Soul Study. *Am J Med*, 2008; 121: 50–57
12. Fehr T, Rickli H: Reply to Sjostrom et al. Cystatin C and creatinine for assessment of kidney function: Differences in pharmacokinetics – and more! *Clin Nephrol*, 2004; 62: 327–28
13. National Kidney F. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis*, 2002; 39: S1–266
14. Levy EM, Viscoli CM, Horwitz RJ: The effect of acute renal failure on mortality. A cohort analysis. *JAMA*, 1996; 275: 1489–94
15. Seeliger E, Sendeski M, Rihal CS, Persson PB: Contrast-induced kidney injury: Mechanisms, risk factors, and prevention. *Eur Heart J*, 2012; 33: 2007–15
16. Duan N, Zhao J, Li Z et al: Furosemide with saline hydration for prevention of contrast-induced nephropathy in patients undergoing coronary angiography: A meta-analysis of randomized controlled trials. *Med Sci Monit*, 2015; 21: 292–97
17. Wang N, Wei RB, Li QP et al: Renal protective effect of probucol in rats with contrast-induced nephropathy and its underlying mechanism. *Med Sci Monit*, 2015; 21: 2886–92
18. Briguori C, Visconti G, Rivera NV et al: Cystatin C and contrast-induced acute kidney injury. *Circulation*, 2010; 121: 2117–22
19. Shlipak MG, Sarnak MJ, Katz R et al: Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Engl J Med*, 2005; 352: 2049–60
20. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E et al: Could neutrophil-gelatinase-associated lipocalin and cystatin C predict the development of contrast-induced nephropathy after percutaneous coronary interventions in patients with stable angina and normal serum creatinine values? *Kidney Blood Press Res*, 2007; 30: 408–15

## Conclusions

In conclusion, our study demonstrated that the change in concentration of Cys C is superior to sCr as a biomarker in the early detection of CIN.

We found that the changed concentration of Cys C is superior to sCr as a biomarker in the early detection of CIN.

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