Use of Cystatin C in the Early Diagnosis of Contrast-Induced Nephropathy

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ABSTRACT

Objective: The aim of this study was to explore the application values of Cystatin C (cys-C) in the early diagnosis of contrast-induced nephropathy (CIN).

Methods: 300 patients, who were performed the percutaneous coronary intervention (PCI) with non-ionotropic or iso-osmotic contrast agent, were selected and detected the levels of Cys C and serum creatinine (Scr) at the 4 time points: before PCI, 24 h, 48 h and 72 h after PCI. Acording to the occurrence of CIN within 72 h of opacification, the patients were divided into the CIN group and non-CIN group, and the changes of Cys C and Scr at different time points between the 2 groups were compared. And the Roc curve was used to compare the early diagnostic values of the 2 indicators towards CIN.

Results: Among the 300 patients, 35 cases occurred CIN, with the incidence rate as 11.67%, the postoperative 24 h Cys C level of the CIN group was significantly increased than the non-CIN group, while the Scr level increased at the postoperative 48 h. The area under the ROC curve of postoperative 24 h Cys C was bigger htan that of Scr (0.898 vs 0.885, P = 0.001). The sensitivity of cys-C as the CIN diagnostic standard was 85.7%, with the specificity as 82.4%.

Conclusions: The post-opacification 24 h serum Cys C concentration exhibited certain significance towards the early diagnosis of CIN, and worthy of clinical generalization.

Keywords: Cystatin C, serum creatine, contrast-induced nephropathy, percutaneous coronary intervention

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INTRODUCTION

The contrast-induced nephropathy refers to the situation that the Scr level increases by 25% or the absolute value increases ≥ 0.5 mg/dl within 72 h of the contrast agent application (1, 2), and the other causes that would affect the renal function have been excluded. With the rapid development of percutaneous coronary intervention therapy (PCI), more and more patients need the use of contrast agent, thus the incidence of CIN has been significantly increased. Currently, the clinics mainly uses the serum creatinine (Scr) to detect CIN, but the sensitivity of Scr towards the early kidney damage is not high, thus there is the require to find a much more sensitive and reliable indicator towards the early identification of CIN (3-5). As a small molecule protein, the cystatin C (Cys C) is produced in vivo by the nucleated cells and would not be interfed by the extrinsic renal factors, thus its production rate is constant, and only excreted through the glomerular filtration (6, 7). Because its molecular weight is greater than the creatinine, and with the positive charge, it makes it easier to reflect the early permeability changes of glomerular filtration membrane, and the much more sensitive marker of renal function than Scr (8, 9). This article aimed to explore the value of Cys C in the early diagnosis of CIN.

SUBJECTS AND METHODS

Subjects

Inclusion criteria: 300 patients with acute coronary syndrome (ACS), who were admitted into the Cardiology Department and performed the PCI treatment from January 2009 to December

2011, were selected, with complete medical records and good therapeutic compliance, including 172 males and 128 females, with the average age as 67.8 ± 12.8 years old. According to the diagnostic criteria of WHO and ACC/AHA, patients with acute myocardial infarction (AMI) were NYHI/KilliP grade I or II, whereas patients with stable angina pectoris after the administration of medications were grade III or IV. The duration of hospitalization is 7 d to 10 d. All patients were given hypotonic or isotonic non-ionic contrast agents. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Tianjin Chest Hospital. Written informed consent was obtained from all participants.

Exclusion criteria: Patients who have an allergy to contrast agents, cardiogenic shock, hypotension, history of kidney resection, malignant tumor, acute or chronic pulmonary disease, severe hepatic or kidney insufficiency, allergy to heparin or low-molecule heparin, coagulation disorders or active gastrointestinal bleeding, severe hypertension without medical interventions, stroke, unknown fever, infection without medical interventions, connective tissue disease, traumatic and surgical history, as well as those who have undergone dialysis or who received any interventional treatment within one week before PCI were excluded.

Study methods

The 300 patients with ACS and undergoing PCI were selected in a prospective manner. All patients were administered the 0.9% NaCl IV 6 h before and after PCI at a rate of 1.0 $mL \cdot kg^{-1} \cdot h^{-1}$ to prevent the hydration. The total dosage of contrast agent used by each patient was recorded. The blood samples were sampled before, 24 h, 48 h and 72 h after PCI to test the SCr and Cys C leves. If the SCr level increased to more than 0.5 mg/dL or 25%, the test

should be repeated 7 d after PCI. The blood sampling kit was provided by the Central Laboratory, and the testing kit for cys-C was manufactured by Dade Behring of Germany.

Selective coronary angiography

PCI was administered through the femoral artery using a 7-French catheter in line with the procedures of Judkin coronary catheterization. 164 patients were given Iohexol, a hypotonic contrast agent with the trade name Omnipaque 350; the average dosage is 124.6 ± 102.3 mL. 136 patients were given Iodixanol, an iso-osmolar contrast agent with the trade name Visipaque 320; the average dosage is 131.6 ± 98.6 mL.

Diagnostic criteria

CIN: According to the guidelines of the European Society of Urogenital Radiology, CIN is defined as kidney insufficiency after using contrast without other predisposing factors or aggravation of pre-existing renal dysfunctions. In addition, a relative increase in SCr by at least 25% from baseline or an increase in the absolute value of SCr level by at least 0.5 mg/dL (≥44.2 μmol/L) within 48 h to 72 h after contrast medium (CM) exposure (Scr peak at 5 d to 7 d, returning to normal at 7 d to 10 d) was considered. However, the effects of cardiac failure, severe arrhythmia, myocardial infarction, and other similar factors were excluded.

CM used in a large dose: The CM dose exceeds the standard limits. The formula for the limited contrast agent is given by: Vmax (mL) = w×5/level of SCr (mL/dL), where w is the weight (kg), and w×5 does not exceed 300.

Cardiac insufficiency: Diagnosed as NYHA grades III to IV according to patients' medical records.

Anemia: Anemia is considered if hemoglobin of an adult male is lower than 12.5 g/dL

and that of an adult female is lower than 11.0 g/dL.

Proteinuria: Result of urine routine shows "+" or more.

Observing indicators and methods

a. The patients' age, gender, blood pressure, dose of contrast medium, blood glucose and lipid levels, and history of primary diseases [including diabetes mellitus (DM), hypertension, cardiac insufficiency, anemia, and proteinuria] were recorded. To confirm who among the patients have CIN, renal function was tested on the first and second day before and after the interventional treatment.

b. Serum cys-C: Immunoassay, such as latex particle-enhanced turbidimetry, was used. The testing kit was purchased from Dade Behring, Germany, and the determination of serum cys-C was performed using a BH13MD1600 automatic biochemistry analyzer (USA).

c. SCr: Picric acid was used to test for creatinine, which was measured using an automatic biochemical analyzer (USA) model: BH13MD1600.

d. BUN: UV-GLDH was utilized to test for BUN, which was measured using automatic biochemical analyzer (USA) model: BH13MD1600.

Statistical analysis

All results were presented as mean ± standard deviation ($\bar{x}$ ±SD), the intergroup comparison used the two-sample independent t-test, and the SPSS13.0 software was used for the statistical process. The Roc curve was used for the diagnostic value determination of Cys c and Scr towards CIN, with $P < 0.05$ considered asthe statistical significance.

RESULTS

Quantity analysis of subjects

All 300 subjects have completed the clinical trial and were provided with the results of the statistical analysis.

Patient characteristics

All the 300 patients (172 males and 128 females) were selected into the analysis, with an average age as 62.57 ± 12.4 years old (16 to 93 years old), among who 69 cases had DM (23.00%), 25 cases had renal insufficiency accompanied with DM (8.33%), 108 cases had hypertension (108/300), 112 cases had hyperlipidemia (37.33%), 42 cases had hyperuricemia, and 79 cases were applied the large dose of CM (25.1%), 26 cases had the cardiac function of grade III or IV (8.67%), 2 cases had anemia (0.67%) and 12 cases had proteinuria (4.00%).

Relevance analysis of between patient's cys-C and SCr

The serum cys-C level of the CIN group was significantly increased 24 h after PCI, while that of Scr did not change significantly within 24 h of opacification, and reached the peak at the 48 h of opacification. Those of the non-CIN group had no significant difference before and after the opacification (Table 1).

Table 1: Comparison of observation indexes between the CIN group and the non-CIN group

index	CIN	Non-CIN
Scr(mg/dL)		
Preoperatively	1.65±0.51	0.94±0.37
24h postoperatively	1.71±0.62	0.95±0.39
48h postoperatively	2.39±0.87	0.95±0.33
72h postoperatively	2.35±0.81	0.94±0.34
Cys C(mg/L)		
Preoperatively	2.15±0.69	1.03±0.37
24h postoperatively	2.97±0.95	1.03±0.34
48h postoperatively	2.99±0.97	1.03±0.38
72h postoperatively	2.81±0.84	1.03±0.36

Comparison of ROC curves of Scr and cys c within 24 h PCI towards the diagnostic values of CIN

24 h after PCI, the Cys C level exhibited the better area under the curve than the Scr level (0.898 vs 0.885, P = 0.001), and the cut-off point value of Cys C was 150 mg/L, with the sensitivity as 85.7% and the specificity as 82.4%; whilt the cut-off point value of Scr was 1.09 mg/dL, with the sensitivity and specificity as 81.3% and 71.4%, respectively (Figure 1).

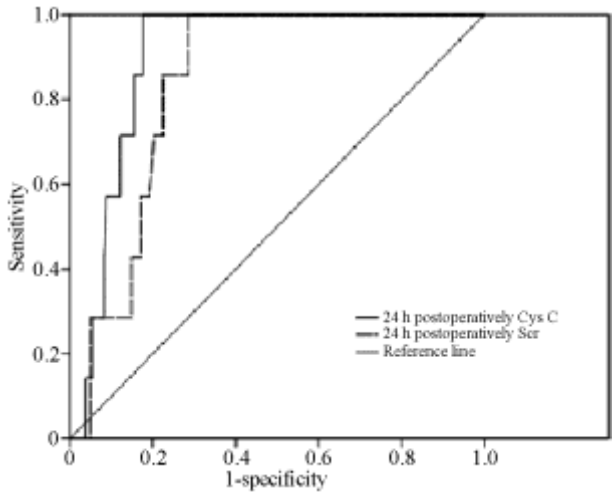

Figure 1. ROC curves of Scr and cys c within 24h PCI towards the diagnostic values of CIN

DISCUSSION

Cys-C is a potent inhibitor of thiol proteases (formerly, post-gamma-globulin, gamma trace). Cys-C was found by Clausen in 1961 in the cerebrospinal fluid and was called gamma trace for its similarity to the architecture and activity of cystatin A and cystatin B. Cys-C was first proposed as a measure of GFR in 1985. Cys-C is a non-glycosylated base protein with 120 amino acid chains, a molecular weight of 13.36×103, and an isoelectric point at pH 9.3. Cys-C is characterized by two disulfide bonds around the c-terminus. The human cystatin locus is located on the short arm of chromosome 20. The genetic sequence can be expressed in most tissues of the body. Cys-C belongs to the type 2 cystatin protease inhibitor family (8).

Cysteine protease includes cathepsin (mainly exists in lysosome) of the papain family and Ca^{2+}-starting protein (mainly exists in cytoplasm) (9) of the Ca^{2+}-starting proteinase family, both of which serve an important function in the metabolism of intracellular peptides and proteins, especially collagen metabolism. The effects of these endopeptidases can be attributed to the thiol of cysteine that is located at the sites of enzyme activation (10). The superfamily has three inhibitory families: type 1 cystatins (stefins), cystatin A and B, which are mainly distributed in the cells; type 2 cystatins, which are a class of cysteine proteinase inhibitors, including cystatin C, D, S, SV, and SA; and type 3 cystatins (kininogens), which are low- and high-molecule kininogens that are mainly distributed in vessels and extracellular spaces (11). According to the study of genetic structure and promoters, all human cells that have a nucleus produce cys-C at a stable rate that is unaffected by inflammatory response, bilirubin, hemolysis, and triacylglycerol and is independent of gender, age, and muscle mass (12). The highest concentration of cys-C was

found in the extracellular fluid (the highest levels are found in semen). The level in the cerebrospinal fluid is higher than that in the blood stream. Cys-C is found in both the saliva and pleural fluid (13). Furthermore, cys-C is found in some cells, such as nerve, thyroid, and islet cells. Cys-C, with a low molecular weight and a high isoelectric point, is removed by glomerular infiltration and metabolized in epithelial cells of the proximal convoluted tubule. This mechanism prevents the reabsorption and secretion of cys-C by the renal tubules (14). Global studies have recently indicated that cys-C is steadily produced in cells with a nucleolus and is unaffected by inflammation and has a low molecular weight, which enables it pass through glomerular infiltration. Given that it is positively charged and has a larger molecular weight, cys-C, with its high sensitivity and specificity, is considered a more precise biomarker of kidney function (as represented by GFR) than serum creatinine (15, 16).

This study conducted a relevance analysis between cys-C and SCr and Ccr. Strong relevance exists among these three indicators with a closer and significantly negative relevance ($r = -0.894$) between Ccr and cys-C with the same direction as SCr ($r = 0.956$). Therefore, cys-C is a more sensitive marker than SCr.

Numerous published papers have introduced the utilization of cys-C in the prediction of changes in GFR. The results of this study indicate that cys-C has high sensitivity and specificity (17, 18). In addition, findings indicate that cys-C is capable of replacing Ccr and SCr as a marker of GFR. These results are the same as those of previously published relevant articles.

In this study, Cys C was set as the observation indicator, aiming to investigate its value towards the early diagnosis of CIN. Certain study had found that the plasma CysC

concentration of patient that had the critical Scr level was the much more effective predictor towards the CIN occurrence, with the sensitivity and specificity as 94.7% and 84.8%, respectively (19). In this study, the Cy s C level of the CIN group was significantly increased with 24 h of PCI, while the Scr level had no significant synchronic change, and the Cys C and Scr leves of the non-CIN group had no statistical significance before and after the opacification, the cut-off point value of Cys C was 1.50 mg/L, with the sensitivity and specificity as 85.7% and 82.4%, respectively, suggesting that the serum Cys C concentrations had a certain value towards the early diagnosis of CIN, better than Scr, and worthy of clinical application.

AUTHORS' NOTE

The authors declare that they have no conflicts of interest or financial ties to disclose.

REFERENCES

1. Nguyen MT, Devarajan P. Biomarkers for the early detection of acute kidney injury. Pediatr Nephrol 2008; **23:** 2151–7.

2. Stacul F, van der Molen AJ, Reimer P, Webb JA, Thomsen HS, Morcos SK et al. Contrast induced nephropathy: updated ESUR Contrast Media Safety Committee guidelines. Eur Radiol 2011; **21:** 2527–41.

3. Tepel M, Aspelin P, Lameire N. Contrast-induced nephropathy: a clinical and evidence-based approach. Circulation 2006; **113:** 1799–806.

4. Droppa M, Desch S, Blase P, Eitel I, Fuernau G, Schuler G et al. Impact of N-acetylcysteine on contrast-induced nephropathy defined by cystatin C in patients with ST-elevation myocardial infarction undergoing primary angioplasty. Clin Res Cardiol 2011; **100:** 1037–43.

5. Ishibashi Y, Yamauchi M, Musha H, Mikami T, Kawasaki K, Miyake F. Impact of contrast-induced nephropathy and cardiovascular events by serum cystatin C in renal insufficiency patients undergoing cardiac catheterization. Angiology 2010; **61:** 724–30.

6. Liu XL, Wang ZJ, Yang Q, Yu M, Shen H, Nie B et al. Plasma neutrophil-gelatinase-associated lipocalin and cystatin C could early diagnose contrast-induced acute kidney injury in patients with renal insufficiency undergoing an elective percutaneous coronary intervention. Chin Med J 2012; **125:** 1051–6.

7. Tanaga K, Tarao K, Nakamura Y, Inoue T, Jo K, Ishikawa T et al. Percutaneous coronary intervention causes increase of serum cystatin C concentration even in the

patients with a low risk of contrast-induced nephropathy. Cardiovasc Interv Ther 2012; **27:** 168–73.

8. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Pawlak K, Mysliwiec M et al. Could neutro-philgelatinaseassociated lipocalin and cystatin C predict the development of contrast-induced nephropathy after percutaneous coronary interventions in patients with stable angina and normal serum SCratininevalues. Kidney Blood Press Res 2007; **30:** 408–15.

9. Koyner JL, Bennett MR, Worcester EM, Ma Q, Raman J, Jeevanandam V et al. Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery. Kidney Int 2008; **74:** 1059–69.

10. Kimmel M, Butscheid M, Brenner S, Kuhlmann U, Klotz U, Alscher DM et al. Improved estimation of glomerular filtration rate by serum cystatin C in preventing contrast induced nephropathy by N-acetylcysteine or zinc--preliminary results. Nephrol Dial Transplant 2008; **23:** 1241–5.

11. Wagener G, Minhaz M, Mattis FA, Kim M, Emond JC, Lee HT et al. Nephrology dialysis transplantationUrinary neutrophil gelatinase-associated lipocalin as a marker of acute kidney injury after orthotopic liver transplantation. Nephrol Dial Transplant 2011; **26:** 1717–23.

12. Laskey WK, Jenkins C, Selzer F, Marroquin OC, Wilensky RL, Glaser R et al. Volume-to-creatinine clearance ratio: a pharmacokinetically based risk factor for prediction of early creatinine increase after percutaneous coronary intervention. J Am Coll Cardiol 2007; **50:** 584–90.

13. Heinrich MC, Häberle L, Müller V, Bautz W, Uder M. Nephrotoxicity of Iso-osmolar Iodixanol Compared with Non-ionic Low-osmolar Contrast Media: Metaanalysisof Random-ized Controlled Trials. Radiology 2009; **250:** 68–76.

14. Toprak O, Cirit M. Risk factors and therapy strategies for contrast-induced nephropathy. Ren Fail 2006; **28:** 365–81.

15. Roy P, Raya V, Okabe T, Pinto Slottow TL, Steinberg DH, Smith K et al. Incidence, predictors, and outcomes of post-percutaneous coronaryintervention nephropathy in patients with diabetes mellitus and normal baseline serum scratinine levels. Am J Cardiol 2008; **101:** 1544–9.

16. Pucelikova T, Dangas G, Mehran R. ContrastInduced Nephro-pathy. Catheter Cardiovasc Interv 2008; **71:** 62–72.

17. Hardiek KJ, Katholi RE, Robbs RS, Katholi CE. Renal effects of contrast media in diabetic patientsundergoing diagnostic or interventional coronary angio-graphy. J Diabetes Complications 2008; **22:** 171–7.

18. Goldstein SL. A novel use for novel acute kidney injury biomarkers: fenoldopam's effect on neutrophil gelatinase-associated lipocalin and cystatin C. Crit Care 2011; **15:** 177.

19. Kato K, Sato N, Yamamoto T, Iwasaki YK, Tanaka K, Mizuno K. Valuable markers for contrast-induced nephropathy in patients undergoing cardiac catheterization. Circ J 2008; **72:** 1499–505.