Valuable Markers for Contrast-Induced Nephropathy in Patients Undergoing Cardiac Catheterization

Koji Kato, MD; Naoki Sato, MD; Takeshi Yamamoto, MD; Yu-ki Iwasaki, MD; Keiji Tanaka, MD; Kyoichi Mizuno, MD*

Background  Contrast-induced nephropathy (CIN) frequently complicates cardiac catheterization, so the objectives of present study were to investigate the usefulness of cystatin C before catheterization and establish a cut-off level for CIN, and to examine the changes in cystatin C and several other markers in patients with and without CIN.

Methods and Results  Prospective study of consecutive 87 patients who underwent elective catheterization: moderate renal disease defined as glomerular filtration rate 30–59 ml·min⁻¹·1.73 m⁻², cystatin C and creatinine (Cr), urinary liver-type fatty acid-binding protein (L-FABP), α₁, β₂ microglobulins, N-acetyl-β-D-glucosaminidase, and microalbumin were measured immediately before, and 1, 2, and 3 days after catheterization. CIN occurred in 18 patients and receiver-operating characteristic analysis showed a higher area-under-the-curve for cystatin C compared with serum Cr (0.933 vs 0.832, p=0.012). At a cut-off level of >1.2 mg/L, cystatin C before catheterization exhibited 94.7% (95% confidence interval: 0.851–1.015) sensitivity and 84.8% specificity for detecting CIN. Cystatin C levels were higher in CIN patients than in those without CIN, even before catheterization (cystatin C: 1.08±0.22 vs 1.36±0.28 mg/L, p=0.007). Urinary L-FABP was increased on days 1 and 2 in patients with moderate renal disease.

Conclusion  Cystatin C was useful for predicting the occurrence of CIN. Urinary L-FABP was the only marker of transient renovascular damage. (Circ J 2008; 72: 1499–1505)

Key Words: Contrast-induced nephropathy; Coronary angiography; Cystatin C; L-FABP; Percutaneous coronary intervention

Contrast-induced nephropathy (CIN) is a recognized complication of both coronary angiography (CAG) and percutaneous coronary intervention (PCI), and is associated with prolonged hospitalization and adverse clinical outcomes. Several risk factors for CIN have been identified: chronic kidney disease (CKD), diabetes mellitus (DM), congestive heart failure, intravascular volume depletion, and using a large amount of contrast media are important predisposing factors. CKD is also a risk factor of CIN. Cystatin C is a cysteine protease inhibitor produced by nearly all human cells and excreted into the bloodstream. It has 120 amino acids with a molecular weight of 13 kDa. The protein is freely filtrated by the renal glomerulus and then metabolized by the proximal tubule, so it is an improved marker of the glomerular filtration rate (GFR) compared with the serum Cr level. Urinary L-FABP is expressed in human proximal tubules and engaged in free fatty acid metabolism, and its excretion reflects stress on the proximal tubules. It has been reported that there is a significant correlation of urinary L-FABP with the extent of tubulointestinal damage.

The goals of present study were to (1) investigate whether or not the serum cystatin C level before cardiac catheterization is a more useful marker of CIN than the serum Cr level, and establish a cut-off level for the determination of CIN, and (2) examine the differences in the changes of the new markers, serum cystatin C and urinary L-FABP, compared with those of the classical markers, urinary α₁ and β₂ microglobulins (MGs), N-acetyl-β-D-glucosaminidase (NAG), and microalbumin (Alb) in patients who underwent elective CAG with and without developing CIN.

Methods

The study was performed from February 2005, through September 2005 at the Cardiac and Intensive Care Unit of Nippon Medical School and included 87 patients (mean 67±11 years, range 43–83, 62 males), who were scheduled for elective cardiac catheterization with or without PCI. This study was approved by the ethical committee of Nippon Medical School and informed consent was given by all patients.
Iomeron® (Eisai Pharmaceutical, Japan), was randomly assigned to one of the three groups: iohexol (350 mg iodine/ml, Omnipaque® (Daiichi Pharmaceutical, Japan) or iomeprol (350 mg iodine/ml, Iomeron®, Eisai Pharmaceutical, Japan) or iomeprol (350 mg iodine/ml, Iomeron®, Eisai Pharmaceutical, Japan) or iomeprol (350 mg iodine/ml, Iomeron®, Eisai Pharmaceutical, Japan) or iomeprol (350 mg iodine/ml, Iomeron®, Eisai Pharmaceutical, Japan). All patients were encouraged to drink if they were thirsty. In patients with LVEF <30% or overt heart failure, the hydration rate was reduced to 0.5 ml·kg⁻¹·h⁻¹. CAG and PCI procedure were performed using standard techniques. Interventional devices were selected by the operators. Patients with acute MI requiring rescue PCI, use of vasopressors prior to the procedure, cardiogenic shock, current peritoneal or hemodialysis, or allergy to contrast media were excluded. A low-osmolal contrast agent, iohexol (350 mg iodine/ml, Omnipaque® (Daiichi Pharmaceutical, Japan) or iomeprol (350 mg iodine/ml, Iomeron®, Eisai Pharmaceutical, Japan), was randomly used. The amount of contrast medium used in each patient was recorded after the procedure. Serum cystatin C and Cr levels, and urinary L-FABP, L-FABP (mg·mg⁻¹·Cr⁻¹) were measured using specific ELISA, as previously described. Serum Cr level was determined by enzymatic assay. Urinary NAG level was determined by spectrophotometry and urinary Alb level was determined by turbidimetric immunoassay. Urinary levels of α1 and β2 MGs were measured using latex agglutination turbidimetry. CIN was defined as an increase of more than 25% from the baseline value of serum Cr, or an absolute increase of at least 0.5 mg/dl (44.2 μmol/L) within 48 h after the administration of contrast medium.

Our first goal was to examine the sensitivity and specificity of the serum cystatin C level for detecting CIN and also to clarify the cut-off value of serum cystatin C using analysis of the receiver-operating characteristic (ROC) curve, while identifying the independent predicting factors for CIN by univariate and multivariate analysis of all patients.

Our second goal was to examine the changes in the serum cystatin C and Cr levels, and the urinary L-FABP, α1 and β2 MGs, NAG, and Alb levels in 31 moderate renal disease patients (22 males, 72±10 years) of the 87 patients who underwent elective CAG with or without PCI. We defined moderate renal impairment as 30×GFR ≤59 ml·min⁻¹·1.73 mm⁻². Estimated GFR was calculated according to the modification of diet in renal disease (MDRD) equation in Japanese: 0.741×175×(serum Cr [mg/dl])⁻¹.154×(age [years])⁻₀.₂₀₃ (if female×0.742) (CKD Practice Guide Booklet).

### Statistical Analysis
The data are expressed as mean ± standard deviation for continuous variables and as frequency (number [%]) for categorical variables. Continuous variables between the patients with and without CIN were compared with the categorical variables. Continuous variables between the patients with and without CIN were compared with the categorical variables.

Table 1 Patients' Characteristics (n=87)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Median (IQR)</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Normal</td>
<td>17</td>
<td>31 (22)</td>
<td>33 ± 7</td>
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<tr>
<td>Mild</td>
<td>31</td>
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GFR, glomerular filtration rate; CIN, contrast-induced nephropathy; CM, contrast medium.

### Study Protocol
All patients scheduled for elective CAG, with or without intervention and gender, height and weight for calculation of the body mass index (BMI), histories of DM, hypertension, and myocardial infarction (MI), were examined. DM was defined as random glucose concentration ≥200 mg/dl, or hemoglobin (Hb) A1c ≥6.5% or under medication on admission. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or under medication. Cardiac echocardiography was performed to evaluate the left ventricular ejection fraction (LVEF). The 0.9% isotonic saline was given intravenously from at least 4 h before the procedure to 12 h after contrast exposure at a rate of 1 ml·kg⁻¹·h⁻¹. All patients were encouraged to drink if they were thirsty. In patient with LVEF <30% or overt heart failure, the hydration rate was reduced to 0.5 ml·kg⁻¹·h⁻¹. CAG and PCI procedure were performed using standard techniques. Interventional devices were selected by the operators. Patients with acute MI requiring rescue PCI, use of vasopressors prior to the procedure, cardiogenic shock, current peritoneal or hemodialysis, or allergy to contrast media were excluded. A low-osmolal contrast agent, iohexol (350 mg iodine/ml, Omnipaque® (Daiichi Pharmaceutical, Japan) or iomeprol (350 mg iodine/ml, Iomeron®, Eisai Pharmaceutical, Japan), was randomly used. The amount of contrast medium used in each patient was recorded after the procedure. Serum cystatin C and Cr levels, and urinary L-FABP, L-FABP (mg·mg⁻¹·Cr⁻¹) were measured using specific ELISA, as previously described. Serum Cr level was determined by enzymatic assay. Urinary NAG level was determined by spectrophotometry and urinary Alb level was determined by turbidimetric immunoassay. Urinary levels of α1 and β2 MGs were measured using latex agglutination turbidimetry. CIN was defined as an increase of more than 25% from the baseline value of serum Cr, or an absolute increase of at least 0.5 mg/dl (44.2 μmol/L) within 48 h after the administration of contrast medium.

Our first goal was to examine the sensitivity and specificity of the serum cystatin C level for detecting CIN and also to clarify the cut-off value of serum cystatin C using analysis of the receiver-operating characteristic (ROC) curve, while identifying the independent predicting factors for CIN by univariate and multivariate analysis of all patients.

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### Statistical Analysis
The data are expressed as mean ± standard deviation for continuous variables and as frequency (number [%]) for categorical variables. Continuous variables between the patients with and without CIN were compared with the
Student’s t-test. The changes of each marker were analyzed by repeated measures analysis of variance with Bonferroni test. Categorical variables are expressed as percentages and analyzed by chi-square or Fisher exact test as appropriate. Analyses of the ROC were conducted for quantitative measurements of the serum cystatin C and Cr levels. To identify the independent predicting factors for CIN, univariate and multivariate logistic regression were used. The potential confounding variables included gender, age, history of MI, hypertension, DM, LVEF, amount of contrast medium, previous therapy (statins, β-blocker, Ca-channel blocker, ACEI/ARB, diuretics), baseline levels of serum cystatin C and Cr, and urinary L-FABP level. Multivariate analyses were performed using the variables that were associated with CIN at the p<0.2 level in the univariate analyses. All tests were 2-sided and p<0.05 was considered statistically significant. All statistical analysis was done with the Statistical Package for Social Sciences version 11.0 (SPSS, Chicago, IL, USA).

**Results**

**Patient Characteristics and Changes in the Markers in Each Group (Tables 1, 2)**

The study group comprised 87 patients (62 males, 71.3%) with a mean age of 67±11 years (range 43–86 years) who underwent elective CAG with or without PCI. CAG was performed for coronary artery disease in 68 patients and congestive heart failure in 19 patients. CIN occurred in 18 patients and was more frequent (45.9%) in the patients with moderate to severe impaired renal function than in the 50 patients with normal to mild renal impairment (2.0%). Iohexiol was used in 42 patients, and iomeprol in 45 patients; CIN occurred in 11 patients who had iohexiol and in 9 patients with iomeprol (p=0.50). The baseline condition of DM was not different between the CIN and without CIN groups (DM patients: 48 (55%); CIN (–), 39 patients: 6.9±1.5% vs CIN (+), 9 patients: 7.0±0.9%, p=0.76).

The changes in the markers in each group are shown in Table 2. Urinary L-FABP levels were significantly increased at 1 day in the mild group, and at 1 and 2 days in the moderate group after CAG compared with the pre-CAG values.

**Cut-Off Value of Cystatin C for Detecting CIN (Fig 1)**

ROC analysis in all patients (n=87, CIN=18) showed a higher area-under-the-curve for serum cystatin C than for serum Cr (0.933 vs 0.832, p=0.05). At a cut-off level of >1.2 mg/L serum, cystatin C exhibited a 94.7% (95% confidence interval: 0.851–1.015) sensitivity and 84.8% in specificity for detecting CIN. Cr, creatinine; CIN, contrast-induced nephropathy.

![Fig 1. Receiver-operating characteristics analysis shows higher area-under-the-curve for serum cystatin C than for serum Cr (0.933 vs 0.832, p=0.05). At a cut-off level of >1.2 mg/L serum, cystatin C exhibited 94.7% (95% confidence interval: 0.851–1.015) sensitivity and 84.8% in specificity for detecting CIN. Cr, creatinine; CIN, contrast-induced nephropathy.](image)

**Table 3 Univariate and Multivariate Logistic Regression Analysis of Variables Related to CIN in All Patients**

| Age (years) | 0.902 | 0.846–0.962 | 0.002* | 0.894 | 0.794–1.006 | 0.062 |
| Sex (male)  | 1.251 | 0.399–3.925 | 0.701 |
| Hypertension| 2.744 | 0.933–8.076 | 0.067 |
| DM          | 1.494 | 0.535–4.147 | 0.441 |
| OMI         | 2.722 | 0.887–8.355 | 0.080 |
| Cr (mg/dl)  | 0.669 | 0.531–0.842 | 0.001* |
| Cystatin C (mg/L) | 0.529 | 0.387–0.724 | <0.001* |
| L-FABP (ng·mg⁻¹·Cr⁻¹) | 0.985 | 0.968–1.003 | 0.985 |
| LVEF (%)    | 1.033 | 0.996–1.072 | 0.077 |
| CM (ml)     | 0.854 | 0.923–1.069 | 0.993 |
| Statins     | 1.620 | 0.552–4.755 | 0.380 |
| β-blocker   | 0.992 | 0.315–3.147 | 0.989 |
| Ca-channel blocker | 2.182 | 0.753–6.319 | 0.150 |
| ACEI/ARB    | 1.587 | 0.571–4.410 | 0.376 |
| Diuretics   | 2.392 | 0.748–7.651 | 0.142 |

* p<0.05.

**Abbreviations:** OR, odds ratio; CI, confidence interval; DM, diabetes mellitus; OMI, old myocardial infarction; LVEF, left ventricular ejection fraction; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker. Other abbreviations see in Tables 1, 2.

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Independent Predictive Factors for CIN (Table 3)

Univariate and multivariate logistic regression analyses performed for all patients revealed that the serum cystatin C level was the only independent variable factor to detect CIN (cystatin C: p=0.028; age: p=0.062). None of the other factors was a valuable factor for CIN. We also performed multivariate analysis for the diabetic patients and in that analysis, cystatin C was also the only predictive marker (cystatin C p=0.021; HbA1c p=0.982).

Patient Characteristics in Moderate Renal Disease (Table 4, Fig 2)

The characteristics of the 31 patients with moderate renal impairment (age: mean 72±10 years, 22 males) are shown in Table 4. In 13 patients, 42.0%, CIN occurred. There were no differences between the groups with and without CIN regarding gender, BMI, previous history of hypertension, DM or MI, reason for CAG, number of PCI and amount of contrast medium, HbA1c or previous therapy, except for age, which was significantly higher in the CIN group (p=0.04). Baseline values of serum Cr, urinary L-FABP, α1 and β2 MGs, NAG, and Alb were not different in both groups; the serum cystatin C level was, however, significantly (p=0.007) higher in the CIN group.

Discussion

The present study demonstrated that (1) the serum cystatin C level was useful for predicting CIN and that its cut-off level was 1.2 mg/L, and (2) urinary L-FABP levels were significantly increased after CAG, suggesting transient proximal tubular damage by contrast medium, although the classical markers were not useful for predicting or detecting CIN.

It is well known that the incidence of CIN partly depends on the serum Cr levels. If the patient has normal renal function, CIN is negligible, even with DM. It should be keep in mind that a borderline serum Cr level does not mean normal renal function (ie, normal GFR). In a retrospective study of 7,586 patients undergoing PCI, 96% of patients had mild to moderate renal insufficiency. In the present study, we denoted moderate renal disease patients as those with GFR from 30 to 59 ml·min⁻¹·1.73 mm⁻², which occurred in 35.7% of the study population, although their serum Cr levels were normal in 87% cases (Cr: mean 1.05±0.28 mg/dl in the group without CIN; 1.05±0.28 mg/dl in the group with CIN, p=0.73). In these patients, CIN occurred in 42%. If the serum Cr level is greater than 4–5 mg/dl, the incidence of CIN is reportedly 50% or more, which is consistent with our results. These findings suggest that GFR is an important factor for the development of CIN. On the other hand, the serum cystatin C concentration might correlate more closely with the GFR than the serum Cr level. Furthermore, the serum cystatin C level is more sensitive in identifying moderate renal insufficiency than the serum Cr level. Taking all these findings together, we speculate that serum cystatin C concentration might be a useful predictive marker of CIN in patients with a borderline serum Cr level, and this was clearly demonstrated by the present study.

The normal range of serum cystatin C is 0.51–0.98 mg/L in a healthy population. There has not been a study regarding the cut-off value of serum cystatin C for detecting CIN. In the present study, we found >1.2 mg/L for serum cystatin C as the cut-off level, and determined the incidence of CIN as 0.028 mg/L.

Table 4 Characteristics of Patients With Moderate Renal Disease

<table>
<thead>
<tr>
<th></th>
<th>CIN (−) (n=18)</th>
<th>CIN (+) (n=13)</th>
<th>p value</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>69±10</td>
<td>76±9</td>
<td>0.04*</td>
</tr>
<tr>
<td>Male sex</td>
<td>13 (72.2%)</td>
<td>9 (69.2%)</td>
<td>0.86</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.0±3.5</td>
<td>22.2±3.7</td>
<td>0.53</td>
</tr>
<tr>
<td>GFR (ml·min⁻¹·1.73 mm⁻²)</td>
<td>51.9±7.5</td>
<td>49.6±8.9</td>
<td>0.45</td>
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Values are mean±SD. *p<0.05, **p<0.01.

BMI, body mass index; CAD, coronary artery disease; PCI, percutaneous coronary intervention. Other abbreviations see in Tables 1–3.
Cystatin C and L-FABP for CIN

Cystatin C to have the highest discriminatory power by ROC. At this cut-off level, the serum cystatin C level exhibited 94.7% sensitivity and 84.8% specificity for detecting CIN before CAG. Another report used a cut-off level of >1.3 mg/L of serum cystatin C for detecting renal dysfunction, which was defined as an iopromide clearance less than 80 ml·min⁻¹·1.73 mm⁻². These values are in excellent agreement with those previously published in various cohorts of cirrhotic (1.25 mg/L), oncologic (1.33 mg/L), and pediatric patients (1.33 mg/L).

The precise pathophysiologic mechanisms responsible for CIN are complex and not completely understood. However, it may involve renal ischemic injury, tubular epithelial cell toxicity, or immunologic reactions. Regarding renal ischemic injury, infusion of contrast medium, with the attendant increases in osmotic load and viscosity, increases the hypoxia of the renal medulla and thus renal free radical production through post-ischemic oxidative stress. Another report indicated that a reduction in renal perfusion caused by a direct effect of contrast medium on the kidney and toxic effects on tubular cells are the main factors in the pathophysiology of CIN. Furthermore, the mechanisms responsible for reduction in renal perfusion involve tubular and vascular events. On the other hand, contrast medium produces marked natriuresis and diuresis, which can activate the tubuloglomerular feedback response leading to vasoconstriction of the glomerular afferent arterioles and causing a decrease in GFR. The present study results suggest that the pre-existing decreasing GFR and its further reduction after administration of contrast might be the factor most involved in the development of CIN.

The present study demonstrated that urinary L-FABP
was a useful marker of renotubular damage caused by contrast medium. In contrast, other renotubular markers (ie, urinary a1 and a2 MGs, NAG and Alb) were not useful in moderate renal disease. Urinary L-FABP is more sensitive than urinary protein in predicting the progression of CKD.\textsuperscript{34} Urinary L-FABP expression in the proximal tubules may be upregulated under tubular stress (eg, tubular ischemia), and urinary excretion of L-FABP from the proximal tubules may increase before the occurrence of cellular structural damage. In contrast, other urinary parameters, such as urinary a1 and a2 MGs, and NAG, increase after cellular structural damage.\textsuperscript{35} On the other hand, one of the mechanisms of CIN is thought to be post-ischemic tubular damage\textsuperscript{36} which is supported by our results, because urinary L-FABP is a good marker of post-ischemic tubular dysfunction.\textsuperscript{37} The present study showed transient increases in urinary L-FABP on the first and second day after the administration of contrast medium and then gradual recovery to baseline values. Increases in both serum Cr and cystatin C tended to be later, suggesting that contrast medium injury begins with tubular damage and then the glomeruli are damaged.

In the present study the most valuable independent factor for detecting CIN was the serum cystatin C level. Recently, the CIN consensus working panel suggested that CKD, DM, volume depletion, nephrotoxic drug use, preprocedural hemodynamic instability, and other comorbidities (eg, anemia, congestive heart failure, hypoalbuminemia) are risk factors, as identified from multivariate analyses.\textsuperscript{37} In the present study, the subjects had relatively moderate renal disease and stable hemodynamics, therefore statistical analysis could not detect the risk factors for CIN other than cystatin C.

Study Limitations
First, our study included a small population and was performed at a single center. Our findings should be confirmed in a large multicenter trial. Second, the estimated GFR was calculated by modified MDRD formula, but this formula may be changed in the near future, because the Japanese Society of Nephrology has decided to create an original equation for obtaining GFR and is undertaking a nationwide project to measure inulin clearance. Therefore, our moderate renal disease population would be slightly different, but basically our results would be same in patients with normal Cr levels.

Conclusions
Cystatin C may be useful for predicting before catheterization the occurrence of CIN, which in the present study occurred in 21% of all patients and in 42% of those with moderate renal disease. The cut-off level was 1.2 mg/L. Urinary L-FABP is the only marker for detecting transient renotubular damage in patients with moderate renal disease.

References