Urinary semaphorin 3A as an early biomarker to predict contrast-induced acute kidney injury in patients undergoing percutaneous coronary intervention

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Abstract

Contrast-induced acute kidney injury (CI-AKI) is a serious complication of diagnostic coronary angiography and percutaneous coronary intervention (PCI). However, the exact pathophysiological mechanisms underlying CI-AKI development are largely unknown. The present study examined whether urinary semaphorin 3A levels predict the development of CI-AKI in patients undergoing PCI. This study enrolled 168 patients with stable angina undergoing elective PCI. Serial urine samples, obtained at baseline and 2, 6, 12, 24, 36, and 48 h post-PCI were analyzed by semaphorin 3A and neutrophil gelatinase-associated lipocalin (NGAL) ELISA kit. AKI was defined as an increase in serum creatinine beyond 50% according to the RIFLE classification system. Receiver operator characteristic (ROC) curve analyses identified optimal semaphorin 3A and NGAL values for diagnosing CI-AKI. CI-AKI occurred in 20 of 168 patients. There were no significant differences in the baseline clinical characteristics and angiographic findings between non-AKI patients group and AKI patients group. Both urinary semaphorin 3A and NGAL levels significantly increased at 2 and 6 h post-PCI. ROC analysis showed that the cut-off value of 389.5 pg/mg semaphorin 3A at 2 h post-PCI corresponds to 94% sensitivity and 75% specificity and the cut-off value of 94.4 ng/mg NGAL at 2 h post-PCI corresponds to 74% sensitivity and 82% specificity. Logistic regression showed that semaphorin 3A levels at 2 and 6 h post-PCI were the significant predictors of AKI in our cohort. Urinary semaphorin 3A may be a promising early biomarker for predicting CI-AKI in patients undergoing PCI.

Key words: Contrast-induced acute kidney injury; Percutaneous coronary intervention; Semaphorin 3A; Neutrophil gelatinase-associated lipocalin; Biomarker

Introduction

Contrast-induced acute kidney injury (CI-AKI) is a serious complication of diagnostic coronary angiography and percutaneous coronary intervention (PCI) (1). CI-AKI often causes adverse clinical outcomes and prolonged hospital stay (2). Studies have proposed that the processes of CI-AKI involve immunologic reactions, ischemic injury, and tubular epithelial cell toxicity (3). Studies also found that there was an increase in hypoxia of the renal medulla and in renal free-radical production through post-ischemic oxidative stress after infusion of contrast medium (4). However, the exact pathophysiological mechanisms underlying CI-AKI development are very complex and largely unknown.

Many risk factors have been suggested to play an important role in the development of CI-AKI. The change of serum creatinine level was well-documented as a risk factor for CI-AKI (5). However, the serum creatinine level does not elevate until glomerular filtration rate (GFR) has decreased by at least 50%, thus, assessment of renal dysfunction according to serum creatinine is not reliable (6). In addition, creatinine clearance value using Cockcroft-Gault formula often overestimates the GFR. As far as we know, the renal markers such as cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), liver fatty acid-binding protein (L-FABP), kidney injury molecule 1 (KIM-1) and interleukin 18 (IL-18) are proposed as potential...
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Patients and Methods

Patients

Our study included 168 patients with stable angina undergoing PCI admitted to the Second Hospital of Tianjin Medical University between January 2014 and June 2016. All the patients gave their written informed consent. This study was approved by the Ethical Committee and the Clinical Studies Committee of the Second Hospital of Tianjin Medical University. The exclusion criteria for this study was as follows: 1) acute myocardial infarction or unstable angina; 2) chronic renal failure (serum creatinine greater than 2.0 mg/dL); 3) history of exposure to contrast.

Methods

Statistical analysis

All the statistical analysis was performed by using GraphPad Prism (version 6.0, USA) and SPSS software (USA). All data are reported as means ± SE. A two-sample
-test or the non-parametric Mann-Whitney U-test was used to compare continuous variables. Chi-square test or Fisher’s exact test were performed to compare categorical variables. To measure the sensitivity and specificity of semaphorin 3A and NGAL for the prediction of AKI, receiver-operating characteristic (ROC) curves were generated and the AUC was calculated. The cut-off value was defined as the closest point to sensitivity: specificity = 1.0 on ROC curve. An AUC-ROC value of 0.90–1.0 indicated excellent, 0.8–0.89 good, 0.70–0.79 fair, 0.60–0.69 poor, and 0.50–0.59 indicated no useful value. Univariate and multivariable logistic regression analysis was performed to assess predictors of AKI. P values less than 0.05 were considered to be statistically significant.

**Results**

**Clinical characteristics of patients with and without AKI before PCI**

Before PCI, we first examined the clinical characteristics of the 168 included patients. As shown in Table 1, there were 116 male and 52 female patients, and the mean age of the whole group was 66.7 ± 3.6 years. Among the included patients, there were 20 patients who developed AKI after PCI procedure, and the AKI rate was 11.9% (20/168). There were no significant differences in the clinical characteristics for age, gender, body mass index, serum creatinine, eGFR, hemoglobin, hemoglobin A1c, left ventricular ejection fraction rate, fasting plasma glucose levels, brain natriuretic peptide level, percentage of hypertension, incidence of diabetes mellitus, and percentage of patients taking diuretics.

| Variables            | Non-AKI (n=148) | AKI (n=20) |
|----------------------|-----------------|------------|
| Gender (male/female) | 100/48          | 16/4       |
| Age (years)          | 67.1 ± 3.3      | 66.2 ± 4.3 |
| BMI (kg/m²)          | 23.2 ± 4.1      | 24.2 ± 3.2 |
| Creatinine (mg/dL)   | 0.96 ± 0.07     | 0.89 ± 0.09|
| eGFR (mL·min/(1.73 m²)| 51.2 ± 11.6    | 47.7 ± 17.1|
| Hb (mg/dL)           | 11.8 ± 1.9      | 12.9 ± 2.5 |
| HbA1c (%)            | 8.3 ± 1.3       | 8.0 ± 1.1  |
| LVEF (%)             | 58 ± 10         | 51 ± 14    |
| BNP (pg/mL)          | 256 ± 57        | 201 ± 43   |
| FPG (mg/dL)          | 118 ± 43        | 123 ± 35   |
| Hypertension (%)     | 102 (68.9%)     | 20 (100%)  |
| DM (%)               | 40 (27.0%)      | 11 (55.0%) |
| Diuretics (%)        | 31 (20.9%)      | 8 (40%)    |

Data are reported as means ± SD or number and percentage. BMI: body mass index; eGFR: estimated glomerular filtration rate; Hb: hemoglobin; HbA1c: hemoglobin A1c; LVEF: left ventricular ejection fraction; BNP: brain natriuretic peptide; FPG: fasting plasma glucose; DM: diabetes mellitus. Statistical analysis was done with the t-test or the non-parametric Mann-Whitney U-test for means and the chi-square test or Fisher’s exact test for proportions. There were no significant differences between groups.

**Angiographic results and lesion features of patients with or without AKI**

The data were compared between the non-AKI group and AKI group, and as shown in Table 2, there were no significant differences in the number of diseased vessels, target vessels, stent diameter, stent length, number of stents used, maximum inflation pressure, and volume of contrast medium.

**Changes in urinary semaphorin 3A and NGAL concentrations after PCI**

We determined the urinary semaphorin 3A concentrations in patients for up to 48 h post-PCI. As shown in Figure 1A, semaphorin 3A levels were significantly elevated at 2 h and 6 h post-PCI procedure, and peaked at 2 h post-PCI in the AKI patients. Levels of semaphorin 3A between AKI and non-AKI groups were no longer significantly elevated at 12 h post-PCI. In the non-AKI patients, the increase of urinary semaphorin 3A level was much less when compared to AKI patients, and the slight increase was not significantly different from baseline (at t = 0 h) (Figure 1A). In addition, we measured the serum levels of creatinine, which is the gold standard biomarker for AKI, and as shown in Figure 1C, the levels significantly increased at 48, 72, and 96 h after PCI in patients with AKI. Furthermore, conventional ROC curves for AKI vs non-AKI were generated for urinary semaphorin 3A at 2, 6, and
The AUCs for the three ROC curves were 0.8576 (P < 0.001), 0.7650 (P < 0.001), and 0.7166 (P < 0.01), respectively (Figure 2). In addition, the sensitivity and specificity for the semaphorin 3A at optimal concentrations were determined at 2 h post-PCI, and the results showed that the cut-off at 389.5 pg/mg of creatinine corresponds to 94% sensitivity and 75% specificity. Furthermore, we compared the other well-studied early biomarker NGAL for AKI post-PCI. As shown in Figure 1B, the changes of urinary NGAL showed a similar pattern to semaphorin 3A: it significantly increased at 2, 6, and 12 h post-PCI and peaked at 2 h. The ROC curves for AKI vs non-AKI were also generated for urinary NGAL at 2, 6, and 12 h, and the AUCs for the three ROCs were 0.632 (P < 0.05), 0.657 (P < 0.05), and 0.619 (P < 0.05), respectively. Further analysis showed that the cut-off value of at 94.4 ng/mg of creatinine for ROC at 2 h post-PCI corresponds to 74% sensitivity and 82% specificity. In addition, the AUC for combined semaphorin 3A and NGAL at 2 h post-PCI was also calculated, and the results showed that the simultaneous occurrence of the 2 urinary biomarkers above the designated threshold did not improve the AUC for the prediction of AKI (Table 3).

**Discussion**

This study demonstrated for the first time that urinary semaphorin 3A is an early predictive biomarker of CI-AKI. Patients undergoing PCI that had CI-AKI showed significantly elevated levels of urinary semaphorin 3A within the first 2 h after PCI, which is much earlier than the rise in serum creatinine by 48–72 h. However, the role of semaphorin 3A in kidney pathophysiology is unknown. Semaphorin 3A is known to have anti-angiogenic effect, but whether semaphorin 3A regulates angiogenesis has not been studied. Since semaphorin 3A was found to regulate cell migration and adhesion, it is likely that it may regulate epithelial cell proliferation and migration, which often occurs immediately after AKI. In animal studies,
semaphorin 3A was found to be localized in distal tubules of the kidney and its levels increased within 3 h after reperfusion of the kidney whereas serum creatinine was significantly raised at 24 h (18). In a more detail animal study, genetic inactivation of semaphorin 3A and pharmacologically based inhibition of semaphorin 3A receptor protected mice from ischemia-reperfusion-induced AKI, and semaphorin 3A was suggested to exacerbate AKI via promoting inflammation and epithelial cell apoptosis (20). Therefore, we may perform future animal studies to

Figure 1. Changes in urinary semaphorin 3A (A), urinary neutrophil gelatinase-associated lipocalin (NGAL) (B) and serum creatinine concentrations (C) at various time points after percutaneous coronary intervention in patients with acute kidney injury (AKI) and without AKI (Non-AKI). Data are reported as means and SD. *P < 0.05 between groups (repeated measures two-way ANOVA).
look into the mechanistic role of semaphorin 3A in CI-AKI development.

NGAL is a 21-kDa, calyx-shaped protein engaged in innate nonspecific immunity mechanisms against bacterial infections and secreted via toll-like receptor activation (21). Various studies have suggested urinary NGAL as a powerful diagnostic tool for CI-AKI. Tasanarong et al. (9), reported urinary NGAL above the threshold of 117 mg/mL measured after 6 h had a sensitivity of 94%, a specificity of 78% and an area under the curve (AUC) of 0.84 for predicting CI-AKI in the patients undergoing elective cardiac catheterization. Further study showed that both urinary and serum NGAL concentrations at 2 and 4 h after PCI, respectively, predicted CI-AKI development (22). In the present study, we used NGAL as a reference biomarker for CI-AKI in comparison with the predictive effect of semaphorin 3A in CI-AKI. We found that both urinary semaphorin 3A and NGAL levels increased significantly at 2, 6, and 12 h after PCI. Further ROC results showed that the AUC of ROC for semaphorin 3A at 2 h after PCI was higher than that of NGAL. ROC analysis of semaphorin 3A at 2 h after PCI showed better predictive sensitivity and specificity when compared to NGAL, which suggests that semaphorin 3A may be a more powerful predictive factor of CI-AKI development in patients undergoing PCI. Indeed, semaphorin 3A has been shown to be a promising biomarker for AKI. Semaphorin was found to predict the development of AKI in liver transplant patients, and the AUC of ROC for semaphorin 3A at 2 h after surgery was 0.631 with an optimal sensitivity of 57% and specificity of 77% (19). In the pediatric AKI, the AUC of ROC for semaphorin 3A at 2 h after surgery was 0.880 with an optimal sensitivity of 81% and specificity of 94% (18). The present study also found comparable results, in which the AUC of ROC for semaphorin 3A at 2 h after PCI was 0.8756 with an optimal sensitivity of 75% and specificity of 82%. Our results may suggest that semaphorin may be a reliable early biomarker to predict CI-AKI in patients undergoing PCI.

The present study has several strengths. We identified a new biomarker for CI-AKI and validated it in human samples. In our study, all subjects started with normal kidney function and low levels of semaphorin in the urine.

Table 3. Predictive features for various combinations of biomarkers at 2 h post-surgery.

| Biomarker or combination | AUC  | Sensitivity | Specificity |
|--------------------------|------|-------------|-------------|
| SEMA (389.5 pg/mg of creatinine) | 0.857 | 0.94 | 0.75 |
| NGAL (94.4 ng/mg of creatinine) | 0.632 | 0.74 | 0.82 |
| SEMA + NGAL | 0.733 | 0.81 | 0.78 |

AUC: area under the ROC curve; SEMA: semaphorin 3A; NGAL: neutrophil gelatinase-associated lipocalin.

Table 4. Prediction of acute kidney injury in univariate and multivariate analysis.

| Predictor (SEMA > 389.5 pg/mg creatinine) | Univariate | Multivariate |
|-----------------------------------------|------------|--------------|
|                                         | Odds ratio (95%CI) | P value | Odds ratio (95%CI) | P value |
| 2 h post-surgery                         | 4.67 (2.55–8.97) | 0.0017 | 3.55 (1.56–7.83) | 0.014 |
| 6 h post-surgery                         | 3.21 (1.78–5.98) | 0.0029 | 2.12 (1.11–5.23) | 0.021 |
| 12 h post-surgery                        | 1.12 (0.76–3.14) | NS         |                    |         |

SEMA: semaphorin 3A; NS: not significant.
Our study also allowed for the temporal definition of changes in semaphorin 3A concentrations in urine after PCI, and a direct comparison with changes in serum creatinine, which is the gold standard for definition of AKI. We also adjusted for the concentration by correcting urinary semaphorin 3A concentrations with urinary creatinine. However, this study also has limitations. It is a single-center pilot study of patients with stable angina undergoing PCI. Thus, these results should be validated in a larger population and multi-center study. Recent studies also showed that renal markers such as cystatin C, NGAL, L-FABP, KIM-1 and IL-18 are proposed as single-center pilot study of patients with stable angina undergoing PCI. Thus, these results should be validated in a larger population and multi-center study. Recent studies also showed that renal markers such as cystatin C, NGAL, L-FABP, KIM-1 and IL-18 are proposed as potential biomarkers for CI-AKI. However, all biomarkers have individual strengths and weaknesses. Given the multifactorial etiologies of AKI, it is unlikely that any single biomarker will suffice. In the future, using a combination of biomarkers might be more accurate for the prediction of CI-AKI.

In conclusion, the present study identified semaphorin 3A as biomarkers for CI-AKI development in patients undergoing PCI. This study may prompt further research into the use of semaphorin 3A along with other currently well-known biomarkers to detect CI-AKI prior to therapeutic strategies in clinical studies. Therapeutic studies based on diagnosis from these biomarkers can be promising in the future.

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References

1. McCullough PA, Wolyn R, Rocher LL, Levin RN, O’Neill WW. Acute renal failure after coronary intervention: incidence, risk factors, and relationship to mortality. Am J Med 1997; 103: 368–375, doi: 10.1016/S0002-9343(97)00150-2.
2. Rihal CS, Textor SC, Grill DE, Berger PB, Ting HH, Best PJ, et al. Incidence and prognostic importance of acute renal failure after percutaneous coronary intervention. Circulation 2002; 105: 2259–2264, doi: 10.1161/01.CIR.0000016043.87291.33.
3. Tepel M, Zidek W. N-acetylcysteine in nephropathy; contrast nephropathy and beyond. Curr Opin Nephrol Hypertens 2004; 13: 649–654, doi: 10.1097/00001552-200411000-00011.
4. Brezis M, Rosen S. Hypoxia of the renal medulla - its implications for disease. N Engl J Med 1995; 332: 647–655, doi: 10.1056/NEJM199509303321006.
5. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem 1992; 38: 1933–1953.
6. Haycock GB. Creatinine, body size and renal function. Pediatr Nephrol 1989; 3: 22–24, doi: 10.1007/BF00859619.
7. Wybraniec MT, Mizia-Stec K. Renalase and biomarkers of contrast-induced acute kidney injury. Cardioren Med 2015; 6: 25–36, doi: 10.1159/000439117.
8. Zhang Z, Lu B, Sheng X, Jin N. Cystatin C in prediction of acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis 2011; 58: 356–365, doi: 10.1053/j.ajkd.2011.02.389.
9. Tasanarong A, Hutayanan P, Piyayotai D. Urinary neutrophil gelatinase-associated lipocalin predicts the severity of contrast-induced acute kidney injury in chronic kidney disease patients undergoing elective coronary procedures. BMC Nephrol 2013; 14: 270, doi: 10.1186/1471-2369-14-270.
10. Manabe K, Kamihata H, Motohiro M, Senoo T, Yoshida S, Iwasaka T. Urinary liver-type fatty acid-binding protein level as a predictive biomarker of contrast-induced acute kidney injury. Eur J Clin Invest 2012; 42: 557–563, doi: 10.1111/j.1365-2362.2011.02620.x.
11. Ling W, Zhaoxiu N, Ben H, Leyi G, Jianping L, Huili D, et al. Urinary IL-18 and NGAL as early predictive biomarkers in contrast-induced nephropathy after coronary angiography. Nephron Clin Pract 2008; 108: c176–c181, doi: 10.1159/000117814.
12. Torregrosa I, Montoliu C, Urios A, Andres-Costa MJ, Gimenez-Garzo C, Juan I, et al. Urinary KIM-1, NGAL and L-FABP for the diagnosis of AKI in patients with acute coronary syndrome or heart failure undergoing coronary angiography. Heart Vessels 2015; 30: 703–711, doi: 10.1007/s00380-014-0538-z.
13. Roth L, Koncina E, Satkauskas S, Cremel G, Aunis D, Bagnard D. The many faces of semaphorins: from development to pathology. Cell Mol Life Sci 2009; 66: 649–666, doi: 10.1007/s00018-008-8518-z.
14. Tufro A, Teichman J, Woda C, Villegas G. Semaphorin 3a inhibits ureteric bud branching morphogenesis. Mech Dev 2008; 125: 558–568, doi: 10.1016/j.mod.2007.12.003.
15. Behar O, Golden JA, Mashimo H, Schoen FJ, Fishman MC. Semaphorin III is needed for normal patterning and growth of nerves, bones and heart. Nature 1996; 383: 525–528, doi: 10.1038/383525a0.
16. He Z, Tessier-Lavigne M. Neurophin is a receptor for the axonal chemorepellent Semaphorin III. Cell 1997; 90: 739–751, doi: 10.1016/S0092-8674(00)80534-6.
17. Villegas G, Tufro A. Ontogenic of semaphorins 3A and 3F and their receptors neurolpins 1 and 2 in the kidney. Mechanc Develop 2002; 119(Suppl 1): S149–S153, doi: 10.1016/S0925-7733(03)00108-4.
18. Jayakumar C, Ranganathan P, Devarajan P, Krawczeski CD, Looney S, Ramesh G. Semaphorin 3A is a new early diagnostic biomarker of experimental and pediatric acute kidney injury. PloS One 2013; 8: e58446, doi: 10.1371/journal.pone.0058446.
19. Lewandowska L, Matuszkiewicz-Rowinska J, Jayakumar C, Oldakowska-Jedynak U, Looney S, Galas M, et al. Netrin-1 and semaphorin 3A predict the development of acute kidney injury in liver transplant patients. PloS One 2014; 9: e107898, doi: 10.1371/journal.pone.0107898.
20. Ranganathan P, Jayakumar C, Mohamed R, Weintraub NL, Ramesh G. Semaphorin 3A inactivation suppresses ischemia-reperfusion-induced inflammation and acute kidney injury. Am J Physiol Renal Physiol 2014; 307: F183–F194, doi: 10.1152/ajprenal.00177.2014.
21. Iyngkaran P, Schneider H, Devarajan P, Anavekar N, Krum H, Ronco C. Cardio-renal syndrome: new perspective in diagnostics. *Semin Nephrol* 2012; 32: 3–17, doi: 10.1016/j.semnephrol.2011.11.002.

22. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Pawlak K, Mysliwiec M, 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: 408–415, doi: 10.1159/000109102.