Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2014, 4(3):157-161

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Significance of combined detection of Cys-C, NGAL and KIM-1 in contrast-induced nephropathy after coronary angiography

Feng Wang[1], Qing Zhao[2], Cheng Peng[3], Junhui Li[1], Changyou Xuan[1] and Niansong Wang[1]

[1]*Department of Nephrology and Rheumatology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China*
[2]*Department of Cardiology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China*
[3]*Suzhou University Medical School, Suzhou, China*

**Drs. Feng Wang, Qing Zhao and Cheng Peng contributed to this work equally.*
***Correspondence**:*
Feng Wang, MD, PhD, zyzwq1030@163.com, Department of Nephrology and Rheumatology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China.
Niansong Wang, MD, PhD, wangniansong2008@163.com, Department of Nephrology and Rheumatology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China.

ABSTRACT

To evaluate the significance of combined detection of Cystatin-C (Cys-C), neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) in the diagnosis of contrast-induced nephropathy (CIN) after coronary angiography (CAG). Sixty-nine patients receiving CAG with high-risk of CIN (risk score not less than 6 or eGFR not more than 60ml/min) were enrolled in this study. Blood and urine samples were collected before and after CAG. Levels of Cys-C, NGAL and KIM-1 were determined by ELISA methods to further explore the specificity and sensitivity for the diagnosis of CIN. The results showed that maximum levels of serum Cys-C (r= 0.798，P<0.001), urine NGAL (r= 0.320, P = 0.007) and urine KIM-1(r= 0.418，P<0.001) post-CAG were positively correlated with the maximum levels of serum creatinine (SCr) post-CAG. The levels of Cys-C at 24h post-CAG (r= 0.840), NGAL at 3h post-CAG (r= 0.367) and KIM-1 (r= 0.458) at 12h post-CAG correlated with maximum SCr post-CAG most significantly. The changes of NGAL（r= 0.271, P = 0.020）and KIM-1（r= 0.230, P = 0.049）were positively correlated with the changes of SCr while Cys-C not. For the diagnosis of CIN, the specificity, sensitivity and AUC of NGAL were 93.9%, 66.7% and 0.659 while the specificity, sensitivity and AUC of KIM-1 were 95.2%, 75% and 0.742 respectively. In conclusion, NGAL and KIM-1 were valuable for CIN diagnosis after CAG. NGAL can indicate the diagnosis of CIN at 3 hours after CAG, while KIM-1 can indicate CIN at 12 hours after CAG more sensitively.

Key words: contrast-induced nephropathy, acute kidney injury, coronary angiography, Cystatin C, neutrophil gelatinase-associated lipocalin, kidney injury molecule-1

INTRODUCTION

As a secondary kidney disease, contrast-induced nephropathy (CIN) is a common iatrogenic renal injury whose incidence in high-risk population is as high as 20% [1-3]. Similar to other secondary kidney diseases, CIN is a significant problem in the world, which may lead to end stage kidney disease even death [4-6]. Serum creatinine (SCr) is the most widely used biomarker for the diagnosis of renal injury, but it is not sensitive to CIN for it usually elevates 1-2 days after renal injury. More and more new markers such as kidney injury molecule-1 (KIM-1) were employed to detect the renal injuries [7].

Neutrophil gelatinase-associated lipocalin (NGAL), one member of the fat carrier protein family with molecular weight of 25KDa, is an acute-phase marker for renal tubular damage [8-10]. The peak level of NGAL appears at 4-6 hour after renal injury and decreases gradually after 24 hours. Furthermore, the sensitivity and specificity of NGAL are both high [11-13]. KIM-1, a transmembrane glycoprotein of the proximal convoluted tubules, is a specific indicator for the injury of the proximal tubules [14]. Levels of KIM-1 in blood and urine rise significantly after ischemia or nephrotoxic injury [15]. The peak level of KIM-1 appears at 6 hour after renal injury, and remains significantly high within 36 hours [16-19]. Cystatin-C (Cys-C), a constitutive protein of nucleated cells, is secreted at low level physiologically and reabsorbed mostly by the proximal tubules after glomerular filtration [17]. According to the literature, Cys-C is more sensitive to assess renal damage than SCr [20, 21]. In order to explore the clinical predictive significance of early biomarkers of CIN, a combined detection of Cys-C, NGAL and KIM-1 was conducted in patients receiving coronary angiography (CAG).

MATERIALS AND METHODS

Study subjects

Sixty-nine high-risk patients including 20 females and 49 males hospitalized at Shanghai Jiao Tong University Affiliated Sixth People's Hospital from August 2009 to January 2010 for CAG or coronary intervention were enrolled. High-risk referred to CIN risk score not less than 6 points proposed by Mehran in 2004 or eGFR $\leq$ 60ml/min. Hypotonic or hypertonic non-ionic contrast media were used in the 69 patients, all of whom received sodium bicarbonate hydration. Before CAG, patients with AMI took 600mg clopidogrel and 300 mg aspirin (ASA) at a draught. After CAG, patients took different anti-thrombotic drugs according to their respective conditions. During the observation period, no additional angiotensin convert enzyme inhibitors (ACEI), angiotensin II receptor blockers (ARB), calcium channel blockers (CCB) or diuretics were used in the two groups, but the original drugs were continued.

Diagnostic criteria and exclusion criteria

Diagnostic criteria: this study adopted the generally recognized definition of CIN [1]. If elevation of SCr is 50% more than the baseline or absolute increase is greater than 44.2μmol/L within 24-72 hours after CAG, the case should be diagnosed with CIN.

Excluding criteria: (i) unstable renal function 1 week before CAG or SCr fluctuation 20% higher than baseline, including SCr instability caused by inadequate perfusion as a result of heart failure after acute myocardial infarction (AMI). (ii) post-CAG heart failure caused by myocardial ischemia-reperfusion injury or cardiogenic shock caused by other reasons. (iii) additional use of ACEI, diuretics, nonsteroid anti-inflammatory drugs (NSAIDS) (except ASA), or other nephrotoxic drugs within 1 week after CAG. (iv) renal replacement therapy, pregnant women, patients with contrast media allergy or poor compliance.

eGFR calculation: according to simplified CG formula: CG-eGFR: Ccr = [(140 - age) × weight × (0.85 female)]/(72 × SCr).

Specimen collection and determination

Serum Cys-C, urine NGAL and KIM-1 within 24 hours before CAG, post-CAG 24h-72h SCr, blood urea nitrogen (BUN), post-CAG 3h, 6h, 12h and 24h urine NGAL, KIM-1, 24h-72h serum Cys-C were detected. Cys-C, NGAL and KIM-1 were measured using ELISA kits (R & D) respectively.

Statistical methods

SPSS 13.0 was employed for data analysis. Various parameters were subject to both single-factor analysis and multi-factor analysis.

RESULTS

Relationship between serum Cys-C and CIN

In this study, there were 63 patients with complete Cys-C data, among which 6 patients were diagnosed with CIN in accordance with the criterion of SCr [1]. Post-CAG Cys-C at 24h, 48h and 72h were positively correlated with maximum SCr (Table 1). In addition, maximum Cys-C was positively correlated with post-CAG maximum SCr (r= 0.798, P<0.001) (Fig. 1). There was no significant correlation between the changes of SCr and Cys-C before and after CAG (r= 0.160, P= 0.182).

If post-CAG Cys-C elevation ≥ 25% (compared with pre-CAG) was taken as the diagnostic criterion for CIN, only 1 in 63 patients was diagnosed with CIN. The specificity of Cys-C for CIN diagnosis was 90.3%, while the sensitivity was 0%, and AUC was 0.491 (Fig. 1).

Table 1 Correlation between Cys-C, NGAL, KIM-1 and SCr

Parameter	r	P
Post-CAG 24h Cys-C	0.840	0.000
Post-CAG 48h Cys-C	0.776	0.000
Post-CAG 72h Cys-C	0.967	0.000
Post-CAG 3h NGAL	0.367	0.002
Post-CAG 6h NGAL	0.359	0.003
Post-CAG 12h NGAL	0.189	0.125
Post-CAG 24h NGAL	0.361	0.003
Post-CAG 3h KIM-1	0.272	0.240
Post-CAG 6h KIM-1	0.276	0.024
Post-CAG 12h KIM-1	0.458	0.000
Post-CAG 24h KIM-1	0.425	0.000

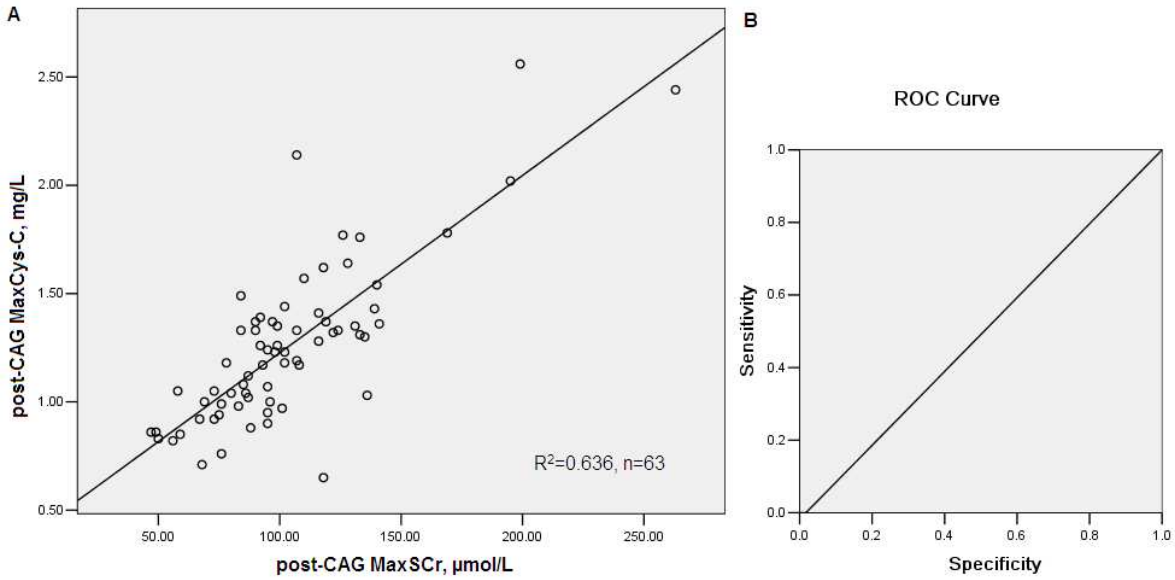

Figure 1 Correlation between maximum Cys-C and maximum SCr post-CAG and ROC curve of Cys-C for CIN diagnosis

Relationship between NGAL and CIN

In this study, all the 69 cases were collected with complete data, among which 6 patients were diagnosed with CIN in accordance with the criterion of SCr [1]. Post-CAG maximum levels of urine NGAL were positively correlated with maximum SCr (r= 0.320 and P= 0.007) (Fig. 2). Furthermore, post-CAG 3h, 6h and 24h NGAL were positively correlated with post-CAG maximum SCr (Table 1). Changes of NGAL were positively correlated with the changes of SCr before and after CAG (r= 0.271, P= 0.020).

With urine NGAL ≥ 300pg/ml as the criterion for the diagnosis of CIN, 3 of the 69 patients were considered as CIN patients. The specificity of NGAL for CIN diagnosis was 93.9%, while sensitivity was 66.7%, and AUC was 0.659 (Table 2 and Fig. 2).

Table 2 Specificity and sensitivity of NGAL and KIM-1 for the diagnosis of CIN

Group	None CIN (n, %)	CIN (n, %)	Total (n)
NGAL<300pg/ml	62, 93.9%	4, 6.1%	66
NGAL≥300pg/ml	1, 33.3%	2, 66.7%	3
Total (n)	63	6	69
KIM-1<3X baseline	59, 95.2%	3, 4.8%	62
KIM-1≥3X baseline	1, 25%	3, 75%	4
Total (n)	60	6	66

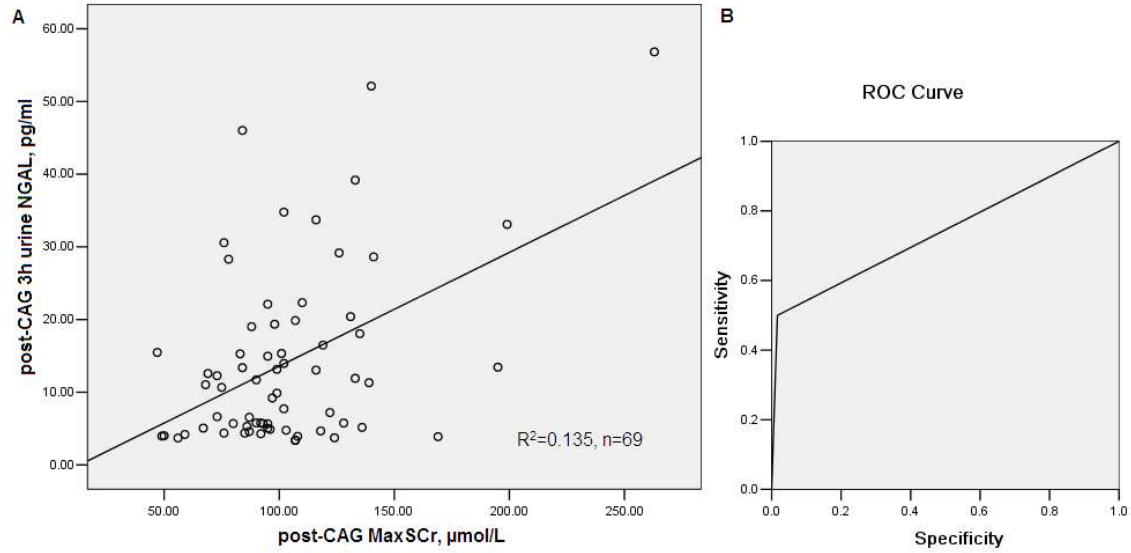

Figure 2 Correlation between post-CAG 3h urine NGAL and post-CAG maximum SCr and ROC curve of NGAL for CIN diagnosis

Relationship between urine KIM-1 and CIN

There were 66 patients with complete KIM-1 data, among which 6 patients were diagnosed with CIN in accordance with the criterion of SCr [1]. Maximum KIM-1 were correlated with post-CAG maximum SCr (r=0.418 and P =0.000) (Fig. 3). Urine KIM-1 at post-CAG 6h, 12h and 24h were linear positively correlated with post-CAG maximum SCr (Table 1). Changes of KIM-1 were positively correlated with the changes of SCr before and after CAG (r=0.230, P =0.049).

With the increase of urine KIM-1 3 times higher than before CAG as the diagnostic criterion, 4 of 66 patients were diagnosed with CIN. The specificity of KIM-1 for the diagnosis of CIN was 95.2%, while sensitivity was 75% and AUC was 0.742 (Fig. 3).

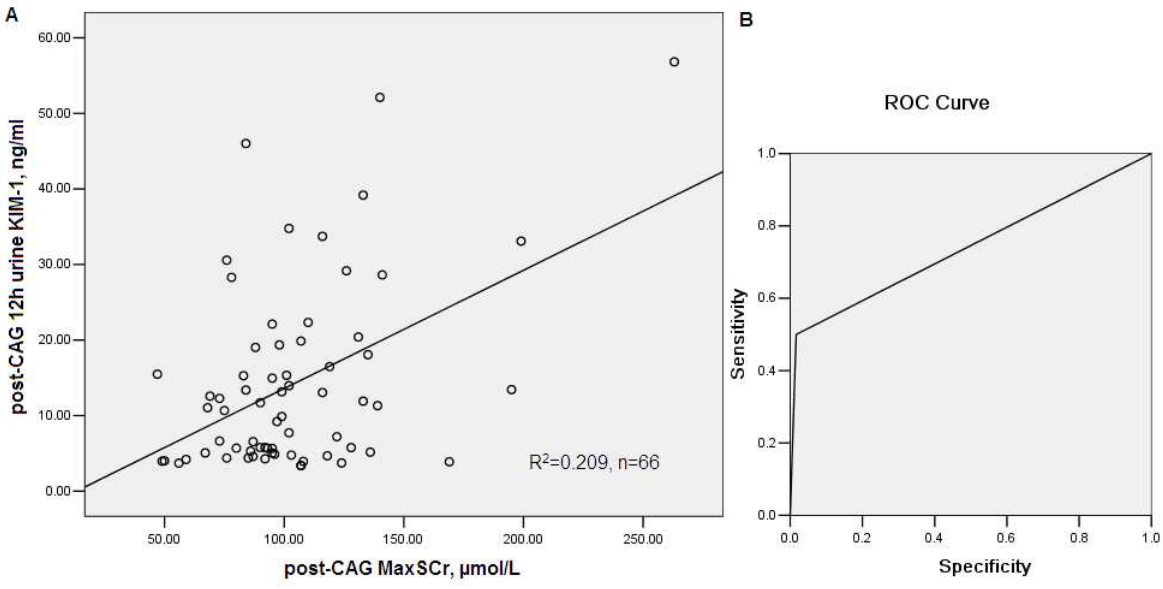

Figure 3 Correlation between post-CAG 12h KIM-1 and post CAG maximum SCr and ROC curve of KIM-1 for CIN diagnosis

DISCUSSION

This study showed that serum levels of Cys-C were positively correlated with the levels of SCr, and the correlation exhibited most significance among the three biomarkers. But it was not an ideal parameter for the diagnosis of CIN. There were linear positive correlations between urine NGAL and SCr and the correlation at post-CAG 3h was the highest. The specificity, sensitivity and AUC of NGAL for CIN diagnosis were 93.9%, 66.7% and 0.659 respectively. Moreover, levels of urine KIM-1 were positively correlated with SCr. There were positive correlations between changes of urine KIM-1 and SCr before and after CAG. The specificity, sensitivity, and AUC of KIM-1 for the diagnosis of CIN were 95.2%, 75% and 0.742 respectively.

The specificity, sensitivity and AUC of NGAL and KIM-1 were all significantly lower than other studies, which may be due to the small subjects size [22]. The AUC of KIM-1 for CIN was not very nice, probably because the definition of CIN cannot effectively define acute kidney injury [23]. In this work, 6 patients met CIN diagnosis criteria of 2010 KDOQI, and the remaining 63 patients did not [22]. In the present study，the diagnosis with new definition did not yield better results, we assumed that the reason might be the fluctuation of SCr was not related to actual kidney injury perfectly in some CIN cases.

In the future, to acquire more significant results it is better to expand study subjects and extend the study period. Randomized controlled experiment would be the best. As the incidence of CIN is relatively not very high, given cost-benefit considerations, new biological indicator test should be conducted among populations with extremely high-risk and high-incidence.

CONCLUSION

Serum Cys-C was well correlated with SCr, but not sensitive to CIN diagnosis. Both NGAL and KIM-1 were positively correlated with SCr, and their AUC for CIN diagnosis were 0.659 and 0.742 respectively. However, Cys-C was not sensitive to CIN diagnosis, although it correlated with SCr.

Acknowledgments
This work was sponsored by the National Natural Science Foundation of China (81100528 and 81270824), the New-100 talent Plan of Shanghai Jiao Tong University School of Medicine and Shanghai talents development fund.

REFERENCES

[1] Solomon R,*Radiol Clin North Am*, **2009**, 47, 783.
[2] Mehran R, E D Aymong, E Nikolsky, Z Lasic, I Iakovou, M Fahy, G S Mintz, A J Lansky, J W Moses, G W Stone, M B Leon,G Dangas,*J Am Coll Cardiol*, **2004**, 44, 1393.
[3] Wang F, J Li, B Huang, Q Zhao, G Yu, C Xuan, M Wei,N Wang,*Ren Fail*, **2013**, 35, 1255.
[4] Shihab S S, H A Al-Hmudi, H S Al-Edani,K H Mahdi,*European Journal of Experimental Biology*, **2014**, 4, 106.
[5] Wang F, T Xing, N Wang,L Liu,*Cytokine*, **2012**, 57, 127.
[6] Kaur H, J Singh, M Verma,K Singh,*European Journal of Experimental Biology*, **2012**, 2, 543.
[7] Hoffmann D, M Adler, V S Vaidya, E Rached, L Mulrane, W M Gallagher, J J Callanan, J C Gautier, K Matheis, F Staedtler, F Dieterle, A Brandenburg, A Sposny, P Hewitt, H Ellinger-Ziegelbauer, J V Bonventre, W Dekant,A Mally,*Toxicol Sci*, **2010**, 116, 8.
[8] Devarajan P,*Cancer Ther*, **2007**, 5, 463.
[9] Mori K,K Nakao,*Kidney Int*, **2007**, 71, 967.
[10] Bachorzewska-Gajewska H, J Malyszko, E Sitniewska, J S Malyszko,S Dobrzycki,*Nephrol Dial Transplant*, **2007**, 22, 295.
[11] Mishra J, C Dent, R Tarabishi, M M Mitsnefes, Q Ma, C Kelly, S M Ruff, K Zahedi, M Shao, J Bean, K Mori, J Barasch,P Devarajan,*Lancet*, **2005**, 365, 1231.
[12] Dent C L, Q Ma, S Dastrala, M Bennett, M M Mitsnefes, J Barasch,P Devarajan,*Crit Care*, **2007**, 11, R127.
[13] Hirsch R, C Dent, H Pfriem, J Allen, R H Beekman, 3rd, Q Ma, S Dastrala, M Bennett, M Mitsnefes,P Devarajan,*Pediatr Nephrol*, **2007**, 22, 2089.
[14] Han W K, V Bailly, R Abichandani, R Thadhani,J V Bonventre,*Kidney Int*, **2002**, 62, 237.
[15] Ichimura T, J V Bonventre, V Bailly, H Wei, C A Hession, R L Cate,M Sanicola,*J Biol Chem*, **1998**, 273, 4135.
[16] Chaturvedi S, T Farmer,G F Kapke,*Int J Biol Sci*, **2009**, 5, 128.
[17] Han W K, G Wagener, Y Zhu, S Wang,H T Lee,*Clin J Am Soc Nephrol*, **2009**, 4, 873.
[18] Han W K, S S Waikar, A Johnson, R A Betensky, C L Dent, P Devarajan,J V Bonventre,*Kidney Int*, **2008**, 73, 863.

[19] Liangos O, W Han, R Wald, M Perianayagam, R Mackinnon, N Dolan, K Warner, J Symes, J Bonventre,B Jaber,*J Am Soc Nephrol*, **2006**, 17, 403A.
[20] Bagshaw S M,R Bellomo,*Curr Opin Crit Care*, **2007**, 13, 638.
[21] Koyner J L, M R Bennett, E M Worcester, Q Ma, J Raman, V Jeevanandam, K E Kasza, M F O'Connor, D J Konczal, S Trevino, P Devarajan,P T Murray,*Kidney Int*, **2008**, 74, 1059.
[22] Mehran R,E Nikolsky,*Kidney Int Suppl*, **2006**, S11.
[23] Nepal M, G H Bock, A M Sehic, M F Schultz,P L Zhang,*Ann Clin Lab Sci*, **2008**, 38, 210.