Biomarkers, 2010; 15(4): 332–339

informa
healthcare

ORIGINAL ARTICLE

Combination of urinary kidney injury molecule-1 and interleukin-18 as early biomarker for the diagnosis and progressive assessment of acute kidney injury following cardiopulmonary bypass surgery: a prospective nested case–control study

Xin-Ling Liang[1,2], Shuang-Xin Liu[1], Yuan-Han Chen[1], Li-Jun Yan[1], Heng Li[3], Hui-Jie Xuan[1], Yong-Zheng Liang[1], and Wei Shi[1]

[1]Division of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, China, [2]Nanfang Medical University, Guangzhou, China, and [3]Division of Laboratory Medicine, Guangdong General Hospital, Guangdong Academy of Medical Sciences, China

Abstract

The aim of this nested case–control study was to assess the combined use of urinary kidney injury molecule (KIM)-1 and interleukin (IL)-18 for acute kidney injury (AKI) after cardiopulmonary bypass surgery (CPB). From a cohort of 122 subjects who underwent CPB, serial urinary KIM-1 and IL-18 concentrations were determined in 30 AKI and 92 non-AKI patients. An increased level of urinary KIM-1 was associated with the occurrence of AKI, whereas an increased level of IL-18 was related to progressive AKI. The combination of these two biomarkers facilitates the early diagnosis and assessment of the likely progression of AKI after CPB.

Keywords: *Acute kidney injury; biomarker; cardiopulmonary bypass; kidney injury molecule-1 (Kim-1); interleukin-18 (IL-18)*

Introduction

Acute kidney injury (AKI) remains a common problem after cardiopulmonary bypass (CPB) with a reported incidence of up to 42%, depending on the population studied and definitions employed (Tuttle et al. 2003, Rosner & Okusa 2006). Preclinical studies have shown that although AKI caused by ischaemia can be reversed by some therapeutic interventions, these approaches should be started very early after the renal insult (Star 1998, Schrier et al. 2004). However, not all cases of AKI are related to adverse prognosis, because they do not all develop to a severe phase (Thakar et al. 2003, Tuttle et al. 2003, Lo et al. 2009). Extensive intervention might be unnecessary in cases of AKI that are unlikely to progress, and could result in over-treatment. Therefore, a biomarker that allows the clinician both to detect the occurrence of AKI at an early stage and to determine whether the case is likely to progress is needed. Unfortunately, although there have been major advances in the development of biomarkers recently, few biomarkers with this bifunctional capability have been validated to date. Thus, it might be more appropriate to use a panel of biomarkers.

The role of interleukin (IL)-18 as an important mediator of ischaemic renal injury, has been observed both in preclinical models and in serial clinical studies (Melnikov et al. 2001, Parikh et al. 2004). A previous study has demonstrated that the detection of urinary IL-18 (uIL-18) and neutrophil gelatinase-associated lipocalin (NGAL) in combination might enable the reliable diagnosis and

The first two authors contributed equally to this work.

Address for Correspondence: Wei Shi, Division of Nephrology, Guangdong General Hospital, 106 Zhongshan Road 2, Guangzhou, Guangdong, 510080, China. Tel.: 86-20-83850849. Fax: 86-20-83850849. E-mail: weishi_gz@126.com

(Received 15 December 2009; accepted 16 February 2010)

ISSN 1354-750X print/ISSN 1366-5804 online © 2010 Informa UK Ltd
DOI: 10.3109/13547501003706558

prognosis of AKI after CPB (Parikh et al. 2006). However, urinary NGAL and IL-18 display their respective actions at different periods after CPB, which limits the use of the combination in clinical practice. Urinary kidney injury molecule (uKIM)-1, which is a transmembrane protein that is highly overexpressed in proximal tubule cells after ischaemia, has served as an early diagnostic indicator of AKI (Han et al. 2002, Liangos et al. 2009). It is noteworthy that uIL-18 and uKIM-1 both display excellent clinical values during a similar time window (Thurman & Parikh 2008), which suggests a basis for their combination as a diagnostic tool.

The aim of this study was to determine the value of combining uKIM-1 and uIL-18 for the early diagnosis and assessment of the likely progression of AKI after CPB.

Subjects and methods

Study design

In this nested case–control study, serial clinical data and samples were collected prospectively for the subjects, who underwent CPB in the cardiac care unit of Guangdong General Hospital between January 2008 and May 2008. Exclusion criteria were: pre-existing chronic kidney disease at stages 3–5, as evaluated by the modified glomerular filtration rate estimating equation for Chinese patients (Ma et al. 2006); potential pulmonary or urinary infections; and the use of nephrotoxic drugs, such as aminoglycosides, vancomycin, non-steroidal anti-inflammatory drugs and Chinese herbs, before or during the study period. In order to avoid potential contrast-induced nephropathy, patients who had undergone imaging with a contrast agent within the 7-day period prior to recruitment were also excluded (Pannu et al. 2006).

Clinical interventions were based on the results of the routine examination rather than results obtained for the urinary markers being investigated. This study was in compliance with the Helsinki Declaration, was approved by the local ethics committee, and written informed consent was obtained for each participant.

Definitions

Levels of serum creatinine were recorded daily as part of routine patient care. Data taken within the 24-h period before the operation were considered to be baseline values. The RIFLE criteria for AKI classifies the patients' grade of AKI on the basis of changes in serum creatinine: RIFLE R ('Risk') denotes an increase in creatinine of 1.5- to 2.0-fold from baseline; RIFLE I ('Injury') denotes an increase in creatinine of 2.0- to 3.0-fold from baseline and RIFLE F ('Failure') denotes an increase in serum creatinine of more than 3.0-fold from baseline (Bellomo et al. 2004).

Given that AKI patients with a progressive trend have a higher mortality than those whose AKI is unlikely to progress and the mortality of patients with RIFLE I is nearly twice that of those with RIFLE R (Bellomo et al. 2004), we defined AKI that developed from RIFLE R to RIFLE I or was more severe than RIFLE R initially as progressive AKI in this study.

Enzyme-linked immunosorbent assay for urinary uIL-18 and uKIM-1

Sequential urine samples were collected at baseline, 2h, 6h and 12 h postoperatively and kept on ice until they were centrifuged at 3000 rpm for 5 min. The supernatant was aliquotted equally into cryovials and then stored at -80°C until the urinary biomarkers and creatinine were measured.

A blinded investigator (H.L.), who was unaware of the clinical characteristics of the subjects, analysed the urine samples. The levels of both uKIM-1 and uIL-18 were measured in duplicate by enzyme-linked immunosorbent assay (ELISA). Urinary KIM-1 was quantified as described previously (Chaturvedi et al. 2009) using a commercially available kit (R&D, Minneapolis, MN, USA). Briefly, the wells of an ELISA plate (Costar, Corning, NY, USA) were coated with the capture antibody, which had been diluted to a working concentration of 0.4 μg ml^{-1} in phosphate-buffered saline (PBS). After incubation overnight at room temperature, each well was washed three times with Wash buffer. The plates were blocked with a 300 μl of reagent diluent (1% BSA in PBS) and incubated at room temperature for 2 h. After three washes as before, 100 μl of standard recombinant human KIM-1, control and a urine sample was pipetted into the designated well for 2 h. After three washes with Wash buffer, the biotinylated goat antihuman KIM-1 detection antibody was added, followed by horseradish peroxidase-conjugated streptavidin and substrate solution. uIL-18 was measured using a commercially available kit (MBL International, Woburn, MA, USA) according to the manufacturer's instructions. Both uKIM-1 and uIL-18 were expressed per milligram of urinary creatinine to correct for differences in urinary concentration. The levels of uKIM-1 and uIL-18 are expressed more frequently in this way than as absolute concentrations, in spite of the fact that the urine creatinine balance of patients with AKI is not in a steady state (Han et al. 2002, 2008, Parikh et al. 2004).

Statistical analysis

Results were expressed as mean ± SD or median (25th, 75th percentile). Differences between the two groups were compared by independent sample analysis.

Comparisons were made by the Student's *t*-tests or the Mann–Whitney *U*-test, as appropriate. Logistic regression was used to analyse the risk of occurrence of AKI or AKI with a progressive trend, and odds ratios (OR) and 95% confidence intervals (95% CI) were derived from the model. To analyse the biomarkers individually, receiver-operating characteristic (ROC) curves were drawn and the areas under the curve (AUCs) were calculated. For joint analysis of the two biomarkers, a fitted multiple logistic regression model was used to give maximum sensitivity and specificity (Table 1) (Han et al. 2009). All *p*-values were two-tailed, and $p < 0.05$ was considered to indicate statistical significance. The statistical procedures were performed using the SPSS (version 13.0; SPSS Inc., Chicago, IL, USA) and Medcalc (version 9.6, Mariakerke, Belgium).

Results

Characteristics of the cohort

In total, 122 eligible subjects of Han Chinese origin (aged 14 years or older) were enrolled. Thirty participants (24.6%) developed AKI after CPB, which included 11 with progressive AKI. Four of the eight patients with RIFLE I and all three patients with RIFLE F received renal replacement therapy. None of the AKI patients were dialysis dependent or died within the 1-month follow-up. Basal levels of age, heart function, urinary biomarkers, serum creatinine, preoperative use of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEI/ARB), CPB time, and surgery indications were compared between the AKI group and the non-AKI group (Table 2).

Changes in urinary biomarkers after CPB

The levels of uKIM-1 in the non-AKI group were unchanged compared with the baseline, whereas the levels in the AKI group were increased 6 h after the operation (Figure 1). In contrast, the levels of uIL-18 in both groups were increased postoperatively at 6 h and 12 h, and no difference was observed between the groups at each time point (Figure 2). Given that at 2 h postoperatively both of the biomarkers were unchanged and did not differ between the two groups, the results of the following analyses at the 2 h time point are not shown.

Table 1. Formula for the fitted multiple logistic regression model for the biomarkers in combination.

For progressive AKI ($n = 11$) vs non-progressive AKI ($n = 111$) combined	
at 6 h	[-3.350 + 0.028 × uKim-1 + 0.014 × uIL-18]
at 12 h	[-4.466 + 0.047 × uKim-1 + 0.023 × uIL-18]

AKI, acute kidney injury; uKIM, urinary kidney injury molecule; uIL -18, urinary interleukin -18.

Relationship between the biomarkers and the occurrence of AKI or a progressive trend

The patients with an increased concentration of uKIM-1 had a higher OR for the occurrence of AKI, whereas those with an increased concentration of uIL-18 did not. In contrast, the patients with a higher concentration of uIL-18 had a higher risk of progressive AKI than those with a lower concentration, but a high concentration of uKIM-1 was unrelated to progressive AKI (Table 3).

Diagnostic performance for early detection of AKI

Table 4 shows the early diagnostic values of uKIM-1 and uIL-18 for AKI. uIL-18 showed a poor diagnostic value for the detection of AKI in this population, which was in accordance with the increased level of uIL-18 in both groups.

To explore the possible reason for the low diagnostic value of uIL-18, we performed a subgroup analysis among

Table 2. Basal characteristics of patients with or without acute kidney injury (AKI).

	AKI ($n = 30$)	Non-AKI ($n = 92$)	*p*-Value
Age (years)	30 (24, 46)	30 (24, 44)	0.730
Heart function (NYHA)	2 (1, 3)	2 (1, 2)	0.095
ACEI/ARB administration	12	38	0.900
Basal biomarkers			
uKIM-1 (ng mg^{-1} creatinine)	1.1 (0.5, 1.5)	0.9 (0.5, 1.3)	0.289
uIL-18 (pg mg^{-1} creatinine)	8.7 (3.5, 25.2)	12.7 (7.3, 17.7)	0.188
Serum creatinine (μmol l^{-1})	90.4 (79.3, 100.2)	89.2 (81.3, 98.7)	0.995
CPB time (min)	129 ± 27	114 ± 21	0.072
Surgery indications			
Valve diseases			
MVR	8	21	0.668
TVR	2	9	0.880
MVR + TVR	8	17	0.335
AVR	4	11	1.000
Congenital heart disease	4	20	0.315
On-pump CABG + valve disease	1	2	1.000
Simply on-pump CABG	3	12	0.904

Values are median (25th, 75th percentile), mean ± SD or absolute numbers. AKI, acute kidney injury; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; uKIM, urinary kidney injury molecule; uIL: urinary interleukin; CPB, cardiopulmonary bypass; MVR, mitral valve replacement; TVR, tricuspid valve replacement; AVR, aortic valve replacement; CABG, coronary artery bypass grafting.

the non-AKI patients. The patients with a higher level of uIL-18 at 6 h had a longer CPB time and higher New York Heart Association (NYHA) classification than those with a lower level, and the patients with a higher level of uIL-18 at 12 h had a higher NYHA classification (Table 5). In addition, the CPB time and level of heart function classification were correlated to the concentration of uIL-18 ($r_s = 0.278$, $p = 0.002$ at 6 h; $r_s = 0.210$, $p < 0.020$ at 12 h; $r_s = 0.358$, $p < 0.001$ at 6 h; $r_s = 0.368$, $p < 0.001$ at 12 h, respectively), which indicated that these non-renal factors might confound the diagnostic value of uIL-18.

Predictive performance for progressive AKI

The predictive performance of uKIM-1 and uIL-18 with respect to the 11 patients with progressive AKI is shown in Table 6. Urinary IL-18 had an excellent predictive value for progressive AKI.

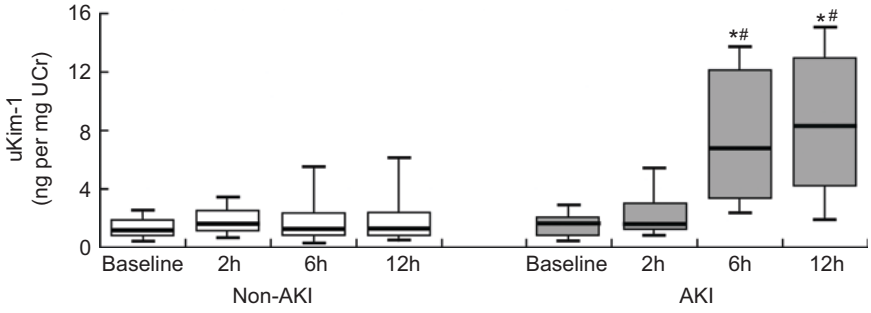

Figure 1. Trends in the level of urinary kidney injury molecule (uKIM)-1, *$p < 0.05$ compared with baseline; #$p < 0.001$ between the two groups at the same time point. Box and whisker plots show the 10th, 25th, 50th (median), 75th and 90th percentile values. AKI, acute kidney injury; UCr, urinary creatinine.

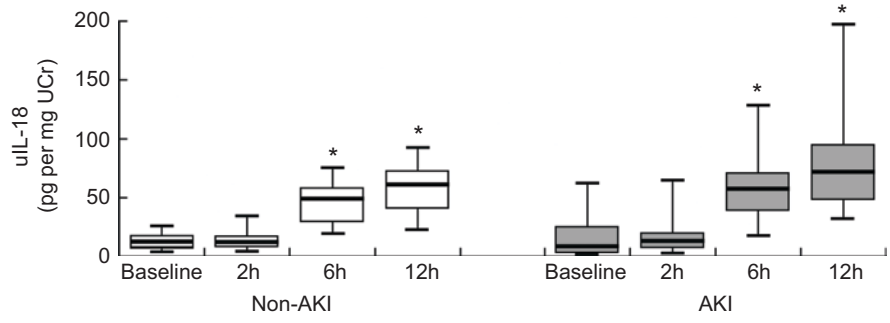

Figure 2. Trends in the level of urinary interleukin (uIL)-18, *$p < 0.05$ compared with baseline. Box and whisker plots show the 10th, 25th, 50th (median), 75th and 90th percentile values. AKI, acute kidney injury; UCr, urinary creatinine.

Table 3. Association between the biomarkers and occurrence or progression of acute kidney injury (AKI).

	OR	95% CI	*p*-Values	Adjusted OR[a]	95% CI	*p*-Values
AKI occurrence						
uKIM-1 at 6 h	1.292	1.138–1.466	<0.001	1.321	1.149–1.518	<0.001
uKIM-1 at 12 h	1.245	1.118–1.386	<0.001	1.291	1.134–1.468	<0.001
uIL-18 at 6 h	1.001	0.993–1.009	0.802	1.001	0.993–1.010	0.774
uIL-18 at 12 h	1.005	0.997–1.013	0.209	1.005	0.997–1.013	0.191
AKI progression						
uKIM-1 at 6 h	1.101	0.900–1.347	0.349	1.116	0.904–1.378	0.307
uKIM-1 at 12 h	1.089	0.919–1.292	0.325	1.112	0.925–1.338	0.258
uIL-18 at 6 h	1.023	1.002–1.044	0. 035	1.023	1.001–1.046	0.045
uIL-18 at 12 h	1.030	1.006–1.054	0.013	1.031	1.006–1.057	0.016

[a]Adjusted odds ratio (OR): adjusted by age, New York Heart Association classification, cardiopulmonary bypass surgery time and with or without administration of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers.

CI, confidence interval; uKIM-1, urinary kidney molecule-1 normalized by urinary creatinine (ng mg^{-1} creatinine); uIL-18, urinary interleukin-18 normalized by urinary creatinine (pg mg^{-1} creatinine).

Early predictive performance of the combination of biomarkers with respect to progressive AKI

The predictive performance of uIL-18 described above was actually based on the late increase in serum creatinine, because of the invalid early diagnostic value of uIL-18. We combined the results obtained for uKIM-1 and uIL-18 at the same time points in a fitted multiple logistic regression model. The predictive performance of the combined biomarkers with respect to progressive AKI at an early stage is shown in Table 7.

Table 4. Performance of uKIM-1 and uIL-18 with respect to the early diagnosis of acute kidney injury (AKI).

	AUC	AUC 95% CI	Sensitivity	Specificity	Positive predictive value[a]	Negative predictive value[a]
uKIM-1 at 6 h						
>1.5	0.881	0.810–0.933	0.933	0.739	0.538	0.971
>2.0	0.881	0.810–0.933	0.767	0.783	0.535	0.911
uKIM-1 at 12 h						
>1.5	0.882	0.812–0.934	0.900	0.728	0.519	0.957
>2.0	0.882	0.812–0.934	0.900	0.783	0.574	0.960
uIL-18 at 6 h						
>50.0	0.614	0.522–0.701	0.600	0.587	0.321	0.818
>60.0	0.614	0.522–0.701	0.433	0.826	0.448	0.817
uIL-18 at 12 h						
>50.0	0.618	0.525–0.704	0.767	0.370	0.284	0.829
>60.0	0.618	0.525–0.704	0.600	0.478	0.273	0.786

[a]Positive predictive values and negative predictive values will vary depending on the prevalence of AKI. The prevalence of AKI for this table is 24.6%.

uKIM-1, urinary kidney molecule normalized by urinary creatinine (ng mg^{-1} creatinine); uIL-18, urinary interleukin-18 normalized by urinary creatinine (pg mg^{-1} creatinine); AUC, area under the receiver operator characteristic curve; CI, confidence interval.

Table 5. Subgroup analysis for the increased uIL-18 in non-acute kidney injury (AKI) patients.

	uIL-18 at 6 h						uIL-18 at 12 h					
	≥50	<50	*p*-Values	≥60	<60	*p*-Values	≥50	<50	*p*-Values	≥60	<60	*p*-Values
NYHA class	2 (1,3)	2 (1,2)	0.024	3 (2,3)	2 (1,2)	<0.001	2 (1,3)	2 (1,2)	0.037	1 (1,2)	2 (1,3)	0.008
CPB time	123 ± 25	108 ± 16	0.001	141 ± 22	108 ± 16	<0.001	113 ± 19	115 ± 23	0.619	110 ± 18	117 ± 24	0.111

The cut-off points for the increased uIL-18 level were referred from Table 4.

uIL-18, urinary interleukin-18 normalized by urinary creatinine (pg mg^{-1} creatinine); NYHA, New York Heart Association; CPB, cardiopulmonary bypass surgery.

Table 6. Performance of uKIM-1 and uIL-18 for predicting progressive acute kidney injury (AKI).

	AUC	AUC 95% CI	Sensitivity	Specificity	Positive predictive value[a]	Negative predictive value[a]
uKIM-1 at 6 h						
>1.5	0.698	0.608–0.778	0.818	0.604	0.170	0.971
>2.0	0.698	0.608–0.778	0.636	0.676	0.163	0.949
uKIM-1 at 12 h						
>1.5	0.702	0.612–0.781	0.818	0.613	0.173	0.971
>2.0	0.702	0.612–0.781	0.818	0.658	0.191	0.973
uIL-18 at 6 h						
>50.0	0.872	0.800–0.926	1.000	0.595	0.196	1.000
>60.0	0.872	0.800–0.926	0.727	0.811	0.276	0.968
uIL-18 at 12 h						
>50.0	0.907	0.840–0.952	1.000	0.369	0.136	1.000
>60.0	0.907	0.840–0.952	1.000	0.505	0.167	1.000

[a]Positive predictive values and negative predictive values will vary depending on the prevalence of progressive AKI. The prevalence of progressive AKI for this table is 9.0%.

uKIM-1, urinary kidney molecule normalized by urinary creatinine (ng mg^{-1} creatinine); uIL-18, urinary interleukin-18 normalized by urinary creatinine (pg mg^{-1} creatinine); AUC, area under the receiver operator characteristic curve; CI, confidence interval.

Table 7. Early predictive performance of the combined biomarkers with respect to progressive acute kidney injury (AKI).

	AUC	AUC 95% CI	Sensitivity	Specificity	Positive predictive value[a]	Negative predictive value[a]
Combined at 6 h						
uKIM-1 > 1.5 and uIL-18 > 50.0	0.883	0.812–0.934	0.818	0.838	0.333	0.838
uKIM-1 > 1.5 and uIL-18 > 60.0	0.883	0.812–0.934	0.353	0.902	0.333	0.902
uKIM-1 > 2.0 and uIL-18 > 50.0	0.883	0.812–0.934	0.774	0.862	0.600	0.862
uKIM-1 > 2.0 and uIL-18 > 60.0	0.883	0.812–0.934	0.364	0.901	0.267	0.901
Combinated at 12 h						
uKIM-1 > 1.5 and uIL-18 > 50.0	0.902	0.835–0.948	0.818	0.321	0.321	0.321
uKIM-1 > 1.5 and uIL-18 > 60.0	0.902	0.835–0.948	0.286	0.316	0.316	0.316
uKIM-1 > 2.0 and uIL-18 > 50.0	0.902	0.835–0.948	0.818	0.856	0.360	0.856
uKIM-1 > 2.0 and uIL-18 > 60.0	0.902	0.835–0.948	0.545	0.901	0.353	0.901

[a]Positive predictive values and negative predictive values will vary depending on the prevalence of progressive AKI. The prevalence of progressive AKI for this table is 9.0%.

uKIM-1, urinary kidney molecule normalized by urinary creatinine (ng mg^{-1} creatinine); uIL-18, urinary interleukin-18 normalized by urinary creatinine (pg mg^{-1} creatinine); AUC, area under the receiver operator characteristic curve; CI, confidence interval.

Discussion

Postoperative AKI, especially cases that show a progressive trend, is associated with an adverse prognosis in patients undergoing CPB surgery. The elevation of serum creatinine, which is a traditional biomarker of AKI, often does not occur until 48–72 h after the initial insult. In addition, an increased level of serum creatinine is not necessarily related to an adverse outcome. As a result, the provision of renal support is very variable in clinical practice (Ostermann & Chang 2009). Thus, it is important to develop biomarkers that allow the identification of AKI with a progressive trend at an early stage. The performances of different biomarkers were compared well in a recent system review (Coca et al. 2008). However, biomarkers that can be measured easily and that enable both early detection and risk stratification for AKI have not yet been validated.

In this prospective nested case–control study, we demonstrated that an increased level of uKIM-1 after CPB is associated with the occurrence of AKI, whereas an increased level of uIL-18 is a risk factor for progressive AKI. Furthermore, our results revealed the performance of uKIM-1 with respect to early diagnosis and that of uIL-18 with respect to the detection of AKI with a progressive trend. We also assessed the performance of the two complementary biomarkers in combination.

The AKI after CPB involves numerous factors, including the type of surgery, CPB time and the preoperative use of ACEI/ARB (Rosner & Okusa 2006, Arora et al. 2008). According to Cleveland Clinic Foundation Acute Renal Failure Scoring System, the patients undergoing coronary artery bypass grafting (CABG) with valve surgery are at higher risk of developing acute renal failure than those undergoing simply CABG or simply valve surgery. However, the combination surgeries form are a relatively low proportion in China (Zheng et al. 2009); there was a 2.4% ratio in the present study. Therefore, the potential effect of surgery type was not analysed in the multivariate models. Also although the CPB time and the preoperative use of ACEI/ARB were comparable between the AKI and non-AKI groups in the univariate model, these factors were associated with AKI occurrence in multivariate models (data not shown), in accordance with the common understanding.

The result of a previous study indicated that uKIM-1 might have some value in predicting an adverse outcome of AKI (Liangos et al. 2007). However, it was based on a population that comprised more severe cases (39% required dialysis, hospital mortality was 24%, and 13% of the survivors were dialysis dependent) and different prognostic outcomes (dialysis or hospital death) in comparison with our study. In the study described herein, the subjects were relatively young with less comorbidity, which contributed to the negative prognostic value of uKIM-1. Moreover, the small sample size in our study might also contribute to this negative value.

There is evidence that uIL-18 might act as an early biomarker of AKI in critically ill patients and during paediatric surgery (Parikh et al. 2005, 2006, Washburn et al. 2008). Surprisingly, uIL-18 appeared to be very sensitive to surgery-related stress in this study, because non-AKI patients with a poor classification of heart function and long CPB time showed increased levels of uIL-18 after CPB, which might attenuate the diagnostic value. Heart failure is associated with an elevated level of plasma IL-18, which is thought to be able to filter through the glomerular basal membrane and be released into the urine (Mallat et al. 2004, Haase et al. 2008). In addition, prior observation has demonstrated that a longer CPB time might contribute to the increase of uIL-18 in non-AKI patients (Haase et al. 2008). Regardless of which explanation is most plausible, these factors represent part of the stress that is responsible for renal injury and leads to AKI. Therefore, it is unsurprising that the AKI and non-AKI groups both had increased

uIL-18 after surgery and it was more likely that AKI in patients with increased levels of uIL-18 would progress than in patients with lower levels.

Our study provided some important information. Firstly, uIL-18 is not a reliable biomarker for early diagnosis. Secondly, on the basis of associations between the biomarkers and AKI, we demonstrated that uKIM-1 could predict the occurrence of AKI and uIL-18 could predict progressive AKI. Finally, we used a multiple logistic regression model to describe the combined values of these two biomarkers, which supported their use in combination. To our knowledge, this is the first time that biomarkers have been combined to allow the simultaneous early diagnosis and risk stratification of AKI.

There are some limitations to our study. It represents the results from a relatively small number of patients in a single centre. Thus, the results will need to be validated in a larger population. In addition, because some exclusion criteria were defined to control confounding factors, we were not able to generalize our results to all cases of AKI after CPB. However, this homogeneous study is possibly a better model for the detection of renal injury in younger subjects in the absence of pre-existing infection and renal disease. Finally, this study lacks the end points of long-term renal adverse outcome and mortality, in spite of a 1-month follow-up. Therefore, the adverse prognosis remains to be validated by evidence from long-term follow-up.

In summary, our results highlight the value of using uKIM-1 and uIL-18 in combination as biomarkers for AKI. Whereas uKIM-1 enables the reliable early detection of AKI, uIL-18 can predict progressive AKI. The use of these two biomarkers in combination facilitates the early diagnosis and assessment of the likely progression of AKI after CPB.

Acknowledgements

This work was supported by a grant from the Guangdong Natural Science Foundation (9151170003000015). We would like to acknowledge the cooperation of Drs Jian Zhuang, Jing-Song Huang, Yi-Qun Ding, Cong Lu and Wei-Ping Xiong (Guangdong Cardiovascular Institute) for their kind assistance in sample collection. We are grateful to Dr Yuantao Hao (School of Public Health, SUN YAT-SEN University) for kind help with the statistical analysis.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Arora P, Rajagopalam S, Ranjan R, Kolli H, Singh M, Venuto R, Lohr J. (2008). Preoperative use of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers is associated with increased risk for acute kidney injury after cardiovascular surgery. *Clin J Am Soc Nephrol* 3:1266–73.

Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. (2004). Acute renal failure – definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 8:R204–12.

Chaturvedi S, Farmer T, Kapke GF. (2009). Assay validation for KIM-1: human urinary renal dysfunction biomarker. *Int J Biol Sci* 5:128–34.

Coca SG, Yalavarthy R, Concato J, Parikh CR. (2008). Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. *Kidney Int* 73:1008–16.

Haase M, Bellomo R, Story D, Davenport P, Haase-Fielitz A. (2008). Urinary interleukin-18 does not predict acute kidney injury after adult cardiac surgery – a prospective observational cohort study. *Crit Care* 12:R96.

Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. (2002). Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int* 62:237–44.

Han WK, Wagener G, Zhu Y, Wang S, Lee HT. (2009). Urinary biomarkers in the early detection of acute kidney injury after cardiac surgery. *Clin J Am Soc Nephrol* 4:873–82.

Han WK, Waikar SS, Johnson A, Betensky RA, Dent CL, Devarajan P, Bonventre JV. (2008). Urinary biomarkers in the early diagnosis of acute kidney injury. *Kidney Int* 73:863–9.

Liangos O, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H, MacKinnon RW, Li L, Balakrishnan VS, Pereira BJ, Bonventre JV, Jaber BL. (2007). Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. *J Am Soc Nephrol* 18:904–12.

Liangos O, Tighiouart H, Perianayagam MC, Kolyada A, Han WK, Wald R, Bonventre JV, Jaber BL. (2009). Comparative analysis of urinary biomarkers for early detection of acute kidney injury following cardiopulmonary bypass. *Biomarkers* 14:423–31.

Lo LJ, Go AS, Chertow GM, McCulloch CE, Fan D, Ordonez JD, Hsu CY. (2009). Dialysis-requiring acute renal failure increases the risk of progressive chronic kidney disease. *Kidney Int* 76:893–9.

Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, Xu JS, Huang SM, Wang LN, Huang W, Wang M, Xu GB, Wang HY. (2006). Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. *J Am Soc Nephrol* 17:2937–44.

Mallat Z, Heymes C, Corbaz A, Logeart D, Alouani S, Cohen-Solal A, Seidler T, Hasenfuss G, Chvatchko Y, Shah AM, Tedgui A. (2004). Evidence for altered interleukin 18 (IL)-18 pathway in human heart failure. *FASEB J* 18:1752–4.

Melnikov VY, Ecder T, Fantuzzi G, Siegmund B, Lucia MS, Dinarello CA, Schrier RW, Edelstein CL. (2001). Impaired IL-18 processing protects caspase-1-deficient mice from ischemic acute renal failure. *J Clin Invest* 107:1145–52.

Ostermann M, Chang RW. (2009). Correlation between parameters at initiation of renal replacement therapy and outcome in patients with acute kidney injury. *Crit Care* 13:R175.

Pannu N, Wiebe N, Tonelli M. (2006). Prophylaxis strategies for contrast-induced nephropathy. *JAMA* 295:2765–79.

Parikh CR, Abraham E, Ancukiewicz M, Edelstein CL. (2005). Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. *J Am Soc Nephrol* 16:3046–52.

Parikh CR, Jani A, Melnikov VY, Faubel S, Edelstein CL. (2004). Urinary interleukin-18 is a marker of human acute tubular necrosis. *Am J Kidney Dis* 43:405–14.

Parikh CR, Mishra J, Thiessen-Philbrook H, Dursun B, Ma Q, Kelly C, Dent C, Devarajan P, Edelstein CL. (2006). Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. *Kidney Int* 70:199–203.

Rosner MH, Okusa MD. (2006). Acute kidney injury associated with cardiac surgery. *Clin J Am Soc Nephrol* 1:19–32.
Schrier RW, Wang W, Poole B, Mitra A. (2004). Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. *J Clin Invest* 114:5–14.
Star RA. (1998). Treatment of acute renal failure. *Kidney Int* 54:1817–31.
Thakar CV, Liangos O, Yared JP, Nelson D, Piedmonte MR, Hariachar S, Paganini EP. (2003). ARF after open-heart surgery: Influence of gender and race. *Am J Kidney Dis* 41:742–51.
Thurman JM, Parikh CR. (2008). Peeking into the black box: new biomarkers for acute kidney injury. *Kidney Int* 73:379–81.
Tuttle KR, Worrall NK, Dahlstrom LR, Nandagopal R, Kausz AT, Davis CL. (2003). Predictors of ARF after cardiac surgical procedures. *Am J Kidney Dis* 41:76–83.
Washburn KK, Zappitelli M, Arikan AA, Loftis L, Yalavarthy R, Parikh CR, Edelstein CL, Goldstein SL. (2008). Urinary interleukin-18 is an acute kidney injury biomarker in critically ill children. *Nephrol Dial Transplant* 23:566–72.
Zheng Z, Li Y, Zhang S, Hu S. (2009). The Chinese coronary artery bypass grafting registry study: how well does the EuroSCORE predict operative risk for Chinese population? *Eur J Cardiothorac Surg* 35:54–8.