MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 3(6) pp. xxx-xxx, June, 2015
Available online http://www.meritresearchjournals.org/mms/index.htm

Original Research Article

NGAL, IL-18, and KIM-1 in urine for early assessment of the risk of developing acute kidney injury in patients undergoing cardiopulmonary bypass

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Abstract

Neutrophil Gelatinase-Associated Lipocalin (NGAL), Kidney Injury Molecule 1 (KIM-1), and interleukin 18 (IL-18) in urine are sensitive quantitative markers for early diagnosis of acute kidney injury (AKI). The purpose of this study was to demonstrate the role of uNGAL, uKIM-1, and uIL-18 for early assessment of renal function. Measurement of structural markers during the first hour's afterextracorporeal circulation (ECC) in patients undergoing cardiopulmonary bypass (CPB) allows detection of AKI much earlier than measurement of serum creatinine (48 hours following surgery). Early diagnosis and risk stratification of developing AKI are critical for adequate therapy. Results were presented as ratios to creatinine in urine allowing better comparability and reliability for variations of instant samples were compensated. Results of thereceiver-operator-characteristic curve (ROC) analysis of uNGAL/uCreat, uKIM-1/uCreat, anduIL-18/uCreat 2 – 6 hours post-ECC demonstrated highest area under the receiver operator characteristic curve for uKIM-1/uCreat- 0.85 (95% CI 0.75 – 0.95, p<0.01). uIL-18/uCreat achieved similar results- AUC 0.83 (95% CI 0.72 – 0.94, p<0.01). The diagnostic performance of uNGAL/uCreat showed AUC 0.78 (95% CI 0.71 – 0.85, p<0.01). Using a combination of structural and functional markers demonstrated the highest predictive value for the risk of developing AKI compensating the shortcomings of independent measurements of single markers.

Keywords: Acute kidney injury, early diagnosis, cardiopulmonary bypass, urinary biomarkers

INTRODUCTION

Acute kidney injury (AKI) is a common and serious complication affecting patients undergoing cardiopulmonary bypass (CPB). Measurement of serum creatinine appears to be unreliable for early detection of AKI due to a number of reasons (Devarajan, 2010) including the influence of non-renal factors such as age, gender, muscle mass and metabolism, medications, hydration and nutrition status, the rate of tubular secretion. Serum creatinine is a late indication of a compromised glomerular filtration rate following important structural changes 2 – 3 days after the moment of injury. In patients undergoing CPB several other factors apply, such as extracellular fluid volume expansion, muscle wasting, and malnutrition (Bonventre and Weinberg, 2003).

During the past decade, an improved understanding of the early pathophysiologic response of the kidney to stress has uncovered a number of genes and proteins that are rapidly induced in the kidney (Devarajan, 2006). Fortunately, some of these proteins are also detected in urine, and are emerging as early non-invasive biomarkers

of AKI revealing novel treatment strategies.

The objective of this study was to demonstrate the performance characteristics of three promising urinary biomarkers for early diagnosis of AKI among adult patients undergoing CPB. These markers include NGAL, KIM-1, and IL-18. Different combinations of urinary biomarkers were explored to point out the optimal urinary panel compensating the shortcomings of independent measurements of single markers.

Study Design, Data Collection and Methods

This multicenter prospective cohort study was conducted between December 2014 and June 2015 at two university hospitals located in Sofia, Bulgaria, and was supported by the Medical University Sofia Grant Commission. All consecutive subjects scheduled to undergo cardiac surgery with CPB. Exclusion criteria were age under 18, pregnancy, surgical intervention during the last 30 days, refusal of giving a written informed consent. The institutional review board of each participating hospital approved the study protocol, and the study was conducted in accordance with the ethical standards laid down in the Declaration of Helsinki of 1975, as revised in 1983.

Medical records were reviewed prospectively and hospitalization data was retrieved, including baseline demographic characteristics, pre-operative laboratory variables, intra-, and postoperative variables, including serum creatinine measurements. The baseline estimated glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study 4-variable equation (Levey et al., 2000).

Urine samples were obtained 2-6 hours after extracorporeal circulation (ECC), and stored in aliquots at -20◦C. Serum creatinine (sCreat) was routinely measured at baseline and 48 hours after surgery (Mehta et al., 2007) AKI and its severity was defined as ≥50% increase in sCreat following the RIFLE (R-risk, I-injury, F-failure, L-loss, E-end stage) criteria (Wim Van et al., 2006). Duration of AKI was defined as the number of days sCreat was ≥50% above baseline.

Laboratory investigators were blinded to clinical outcomes. uNGAL was assayed using a human-specific commercially available ELISA (BioVendor, Czech Republic). uKIM-1 was assayed using a commercially available ELISA (BioVendor, Czech Republic), and uIL-18 was assayed using commercially available ELISA (RayBio®, Norcross GA). Serum and urine creatinine were measured with an enzymatic method using biochemical analyzer Abbott Architect ci4100.

The analysis of the results included patients who had sCreat measurement at baseline, a single-point measurement of uNGAL, uKIM-1, uIL-18, and uCreat 2-6 hours after ECC, and sCreat measurement at 48 hours after surgery. Demographic, baseline measurements and clinical outcomes were compared between AKI and non-AKI patients using Fisher's exact test. Univariable logistic regression was used to assess the discriminative ability of urinary biomarkers to predict AKI. Receiver operating characteristic (ROC) curves were generated for each biomarker at the single-point measurement. The areas under the curve (AUC) were compared between biomarkers using the methods of DeLong (DeLong et al., 1988). In addition the results of the suggested urinary biomarkers were presented as ratios to uCreat, allowing better comparability and reliability for variations of instant samples were compensated. Combinations of two or three urinary biomarkers were suggested to demonstrate their enhanced performance compared to independent measurements of single markers.

RESULTS

80 patients, at mean age of 65 years were enrolled in the study. 34% were females. Mean estimated GFR at baseline was 86 ml/min/1.73 m^2, calculated using MDRD (The Modification of Diet in Renal Disease) formula. Serial measurements of sCreat showed stable values and none of the patients had indications of a preoperative AKI. In all participants, cardioplegia was achieved, after external aortic cross-clamping, through antegrade and retrograde cold blood diastolic cardioplegic arrest. Cardiac function was regained at the end of CPB either spontaneously by reperfusion with warm, circulating blood or through defibrillation.

27.5% of the patients had ≥ 50% increased values of sCreat 48 hours after ECC defined as AKI.

All urine samples were assayed for 2-6-hour post-ECC uNGAL, uKIM-1, uIL-18, and uCreat and ratios of structural biomarkers to creatinine were calculated.

The results of ROC analysis of uNGAL/uCreat, uKIM-1/uCreat и uIL-18/uCreat 2-6 hours post-ECC (Table 1) revealed the highest AUC for uKIM-1/uCreat- 0.85 (95% CI 0.75 – 0.95, $p<0.01$). The results for uIL-18/uCreat were assessed as comparable- AUC 0.83 (95% CI 0.72 – 0.94, $p<0.01$). The performance of uNGAL/uCreat revealed AUC 0.78 (95% CI 0.71 – 0.85, $p<0.01$).

Table 2 presents the optimal cut-off values of the three calculated ratios with their sensitivity, specificity, positive and negative predictive value 2-6 hours post ECC.

In the course of the current study the predictive values of three urinary markers or early detection of AKI were compared as well as their calculated ratios to urine creatinine. A single-point measurement 2-6 hours after ECC among patients undergoing CPB pointed out uKIM-1, respectively uKIM-1/uCreat as a marker with excellent performance characteristics. As a marker for tubule dedifferentiation uKIM-1 revealed excellent sensitivity and specificity for early diagnosis of ischemia-reperfusion conditioned AKI. uIL-18 as a mediator of ischemic injury through apoptosis of tubular epithelial cells and its ratio to

Table 1. AUC- area under the ROC curve for early AKI biomarkers in urine, measured 2-6 hours after ECC.

Predictive variable	AUC	95% CI
uKIM-1/uCreat	0.85	0.75 – 0.95
uIL-18/uCreat	0.83	0.72 – 0.94
uNGAL/uCreat	0.78	0.71 – 0.85

*AUC- area under the curve; CI- confidence interval; AKI- acute kidney injury; ECC- extracorporeal circulation

Table 2. Characteristic performance of calculated ratios for uNGAL/uCreat, uKIM-1/uCreat и uIL-18/uCreat based on the optimal cut-off value 2-6 hours post ECC.

Predictive variable	Optimal cut-off cvalue	Sens. (%)	Spec. (%)	Positive predictive value (%)	Negative predictive value (%)
uKIM-1/uCreat	0.310 ng/mg	95	69	23	99
uIL-18/uCreat	56.5 pg/mg	90	76	29	94
uNGAL/uCreat /females/ uNGAL/uCreat/males/	115 ng/mg 92 ng/mg	72	58	13	96

Table 3. Combinations of calculated ratios for urinary markers to urine creatinine and the change in their prognostic performance compared to uNGAL/uCreat single determination.

		T = 2 – 6hours
Combinations of biomarkers	p<0.01	ΔAUC
uNGAL/uCreat	yes	
uIL-18/uCreat	yes	0.09
uNGAL/uCreat	yes	
uKIM-1/uCreat	yes	0.12
uNGAL/uCreat	yes	
uKIM-1/uCreat	yes	0.15
uIL-18/uCreat	yes	

ΔAUC = Improvement in AUC when compared with uNGAL/uCreat single determination

urine creatinine also performed as a reliable prognostic marker.

uNGAL had previously been shown to have an excellent diagnostic performance, particularly among children undergoing cardiac surgery (Michael et al., 2008). uNGAL and uNGAL/uCreat in the patients cohort enrolled in the current study revealed less impressive results, probably due to differences in patient characteristics including the burden of co-morbidities in adults undergoing cardiac surgery, especially cardiovascular and chronic kidney disease.

Single measurements of urinary markers have reliable predictive value for developing AKI. In most of the cases their disadvantages are related to the hemodynamic effect of volume infusions in concrete patients cases combined with the influence of co-morbidities among adult patients cohort. It makes apparent that there is no single perfect AKI biomarker. Recent studies have explored the possibility of generating panels of biomarkers to provide the best diagnostic and prognostic information in a context-specific manner (Heise et al., 2011; Koyner et al., 2010). We combined uNGAL/uCreat ratio with other urinary biomarker ratios and evaluated the performance of these combinations. Panels of two or three calculated ratios were compared to uNGAL/uCreat single determination. AUC improvement is summarized in Table 3. The best predictive ability was obtained when all three ratios were included.

DISCUSSION

The results of the current study confirmed that NGAL, KIM-1, and IL-18 in urine are predictors of AKI in adult post-CPB settings. Calculated ratios of urinary markers to urine creatinine allowed better comparability and

reliability thus compensating some effective modifiers of their performance, such as age (Haase et al.,2009), gender, volume infusions in the course of therapy, etc. To our knowledge, the current study is the first to assess the elevation of uNGAL/uCreat, uKIM-1/uCreat, and uIL-18/uCreat alone and in combination for early detection of AKI 2-6 hours in post-CPB adult patients.

Cardiac surgery involving CPB is the most frequent major surgical procedure performed in hospitals worldwide. The pathogenesis of post-CPB AKI includes ischemia-reperfusion injury, exogenous toxins such as non-steroid anti-inflammatory drugs, endogenous toxins such as iron released from hemolysis, and inflammation and oxidative stress from contact with bypass circuit, surgical trauma, and intra-renal inflammatory responses. Clinical models including age and CPB time predict AKI with reasonable certainty. Measurement of early urinary markers improves the predictive value of these models and presenting the results as ratios to urine creatinine facilitates the assessment of renal implication compensating the influence of various extra-renal variables in a heterogeneous patient's cohort.

Human NGAL is the most studied of the three suggested markers for early detection of AKI. In cross-sectional study of adults with established AKI from varying etiologies, a marked increase in urine NGAL was documented when compared to normal controls (Mori et al., 2005). uNGAL levels correlated with serum creatinine, and kidney biopsies in patients with AKI showed intense accumulation of immunoreactive NGAL in cortical tubules, confirming NGAL as a sensitive index of established AKI. KIM-1 is a transmembrane protein that is not expressed in healthy kidney but is specifically upregulated in dedifferentiated proximal tubule cells after ischemic AKI. It has been identified as a phosphatidylserine receptor that transforms epithelial cells into phagocytes by recognizing cell surface-specific epitopes expressed by apoptotic tubular epithelia (Ichimura et al., 2008). A proteolytically processed extracellular domain of KIM-1 is detectable in the urine soon after AKI. IL-18 is a pro-inflammatory cytokine and a known mediator of inflammation that is induced in the proximal tubule, and activated by caspase-1 (Melnikov et al., 2001). It is a pro-apoptotic molecule that has the potential of worsening the functional consequences of AKI. While very small amounts are present in normal urine, its concentration is dramatically increased soon after AKI in animal and human models.

In our study we made a single-point measurement of uNGAL, uKIM-1, uIL-18, and uCreat between 2 and 6 hours after ECC in patients undergoing cardiac surgery involving CPB. We also measured 48 hours post-CPB serum creatinine to delimitate AKI from non-AKI patients following the RIFLE criteria. We found that the levels of uKIM-1, respectively uKIM-1/uCreat have the highest predictive value for developing AKI. The results for uIL-18 and uIL-18/uCreat revealed comparable predictive value. uNGAL and uNGAL/uCreat showed worse performance which could be related to confounding pathophysiological states affecting NGAL concentrations. In order to provide optimal performance of urinary markers for early detection of AKI we analyzed and compared combinations of two and three markers and obtained a significantly increased sensitivity and specificity of the panel including all three ratios.

This study is the first to compare the diagnostic performance of uNGAL, uKIM-1, uIL-18 and their calculated ratios to urine creatinine for early detection of AKI in Bulgarian patients undergoing cardiac surgery involving CPB. It is also the first one to suggest an optimal combination of uNGAL/uCreat, uKIM-1/uCreat, and uIL-18/uCreat for an enhanced prediction of AKI.

ACKNOWLEDGEMENTS

The current study was supported by Medical University-Sofia Grant Commission (contract No4/2014, research project No 19-302/15.01.2014)

Conflict of Interest

The authors declare that there is no conflict of interests.

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