Paediatrica Indonesiana

VOLUME 55 March • 2015 NUMBER 2

Original Article

Relationship between serum cystatin-C and urinary neutrophil gelatinase-associated lipocalin in septic children

Jose Mandei, Elisa Iskandar, Adrian Umboh, Hesti Lestari

Abstract

Background Sepsis may lead to acute kidney injury (AKI) in patients treated in pediatric intensive care units (PICU). Currently, serum creatinine is used as a biomarker for the diagnosis of AKI. However, it is not a sensitive nor specific test for AKI. The scarcity of biomarkers leads to delays in the diagnosis and treatment of AKI. Serum cystatin-C (sCys-C) and urinary neutrophil gelatinase-associated lipocalin (uNGAL) are potential biomarkers that look promising for early diagnosis of AKI.

Objective To identify the relation of cystatin-C and NGAL in children with sepsis.

Methods Serum cystatin-C and uNGAL were measured on septic patients aged one month to 12 years. The diagnoses of sepsis were based on the *2002 International Pediatric Sepsis Concensus*. Patients were admitted to the Pediatric Intensive Department of the Prof. Dr. R. D. Kandou Hospital, Manado from January to June 2013. The exclusion criteria were patients with trauma, burns, severe dehydration, malnutrition, obesity, and history of renal diseases. Data analyses included descriptions for the characteristic data and Pearson's coefficient correlation. A P value of 0.05 was considered to be statistically significant. Data were analyzed with *SPSS software for Windows version 21*.

Results Thirty-eight patients met the inclusion criteria, of whom 23 were male and 15 were female. Their mean age was 22.6 (SD 32.24) months, with overweight in 2 children, good nutrition in 25 children, and under nutrition in 11 children. An increased level of sCys-C was found in 22 children and an increased level of uNGAL was found in 19 children. Serum cystatin-C was significantly correlated to uNGAL in septic patients ($r=0.614$; $P<0.01$).

Conclusion There is a positively correlated relationship between sCys C and uNGAL in septic children. Increased sCys C is associated with increased uNGAL in septic children. **[Paediatr Indones. 2015;55:83-6.]**

Keywords: *sepsis, serum cystatin C, urinary NGAL*

Acute kidney injury (AKI), formerly known as acute renal failure, is a common clinical problem in critically ill patients.[1-3] Sepsis is now recognized as the most important contributing factor to AKI in this patient population.[3] For example, acute renal failure occurs in approximately 19% of patients with moderate sepsis, in 23% with severe sepsis, and in 51% with septic shock when blood cultures are positive.[1,2] In the US, an estimated 700,000 cases of sepsis occur each year, resulting in more than 210,000 deaths.[4] The combination of acute renal failure and sepsis is associated with 70% mortality, as compared to 45%

From the Department of Child Health, Sam Ratulangi University Medical School/Prof. Dr. R. D. Kandou Hospital, Manado.

Reprint requests to: Jose Mandei, Department of Child Health, Sam Ratulangi University Medical School, Prof. Dr. R. D. Kandou Hospital, Jl. Raya Tanawangko, Manado, Indonesia. Tel. +62 (431) 821652. Fax. +62 (431) 859091. E-mail: mandeijose.81371@gmail.com.

mortality among patients with acute renal failure alone. Thus, the combination of sepsis and acute renal failure constitutes a particularly serious medical problem in the US.[5]

In clinical practice, a reliable marker to assess the severity of AKI would be a valuable asset for the management of patients with septic AKI in critical care settings. Among potential biochemical markers, certain studies have explored urine neutrophil gelatinase-associated lipocalin (uNGAL) and serum cystatin-C (sCys-C) as diagnostic and prognostic indices for AKI. Human NGAL was originally identified as a 25-kDa protein covalently bound to gelatinase from neutrophils.[5-7] Although NGAL is expressed only at very low levels in several human tissues, it is markedly induced in injured epithelial cells, including in the kidney.[8] Serum cystatin-C is simply a marker of glomerular filtration rate (GFR).[9] The aim of this study was to assess for an association between cystatin-C and urinary NGAL in children with sepsis who were treated in our pediatric emergency care unit.

Methods

We conducted a cross-sectional study in the pediatric emergency care unit of Prof. Dr. R. D. Kandou Hospital, Manado, from January to June 2013. The inclusion criteria were patients aged one month to 12 years with the diagnosis of sepsis based on the *2002 International Pediatric Sepsis Concensus*.[10] Subjects' parents provided informed consent. We excluded children with trauma, burns, severe dehydration, malnutrition, obesity, or history of renal diseases.

Baseline characteristics of subjects were collected after enrollment. Blood specimens for routine hematology tests, serum creatinine levels, blood culture, and sCys-C levels as well as urine specimens for uNGAL were obtained at the time of admission to the PICU.

Acute kidney injury was defined based on modified risk, injury, failure, loss of kidney function, and end-stage kidney disease (RIFLE) classification as follow: (1) AKI pRIFLE risk : estimated creatinine clearance (eCCL) decrease by 25% or urin out put (UOP) < 0.5mL/kg/h for 8 hrs, (2) AKI pRIFLE injury: eCCL decrease by 50% or UOP < 0.5mL/kg/h for 16 hrs, (3) AKI pRIFLE failure: eCCL decrease by 75% or < 0.3mL/kg/h for 24 hrs or anuric for 12 hrs, (4) AKI pRIFLE loss persistent failure > 4 weeks, (5) AKI pRIFLE end stage: persistent failure > 3 months.

Urine specimens were collected in dedicated sterile vials, centrifuged at 2,000 rpm for 5 min, and supernatants stored at –80^{0}C. Blood specimens for sCysC were collected in sterile vials, centrifuged at 3,600 rpm for 10 min, and supernatants stored at –80^{0}C. Urinary NGAL was measured with the *ARCHITECT system* by Abbott (normal range of uNGAL 0.4-72 ng/mL). Serum Cys-C was measured in the *Prodia Laboratory* (normal range of sCys-C : girl 0.5-0.9 mg/L, boys 0.6-0.9 mg/L). Routine hematology tests, serum creatinine levels, and blood cultures were done in the hospital laboratory.

Table 1. Subjects' characteristics

Characteristics	(n=38)
Mean age (SD), months	22.61 (32.24)
Gender, n (%)	
Male	23 (60.5)
Female	15 (39.5)
Nutritional status, n (%)	
Overweight	2 (5.3)
Good nutrition	25 (65.8)
Under-nutrition	11 (28.9)
Mean temperature (SD), ^{0}C	38.518 (1.24)
Mean pulse (SD), x/m	144 (19.59)
Mean respiration (SD), x/min	54.58 (12.13)
Mean systole (SD), mmHg	93.16 (7.39)
Mean diastole (SD), mmHg	60.26 (6.77)
Acute kidney injury, n (%)	22 (57.89)
High sCys-C level, n (%)	19 (50.00)
High u-NGAL level, n (%)	23 (60.53)

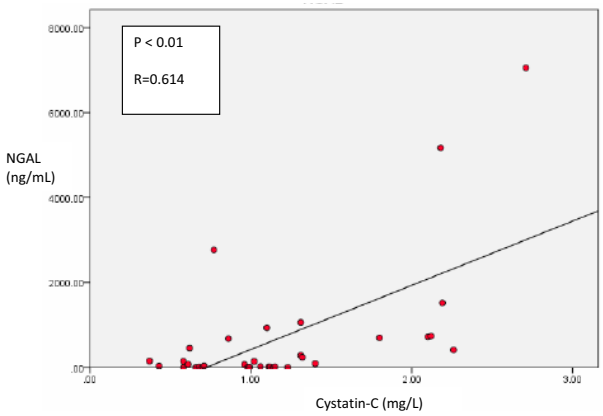

Figure 1. Correlation between serum Cys-C and uNGAL

Data was analyzed by description for the characteristic data and Pearson's correlation coefficient. A P value of 0.05 was considered to be statistically significant.

Results

There were 38 septic patients involved in this study. The characteristics of subjects are shown in **Table 1**.

Pearson's correlation analysis revealed a positive correlation between increased serum Cys-C and increased uNGAL in septic patients ($r=0.614$; $P<0.01$) (**Figure 1)**.

Discussion

Studies on NGAL as an early AKI biomarker have shown promising results both in adults and children, since uNGAL levels rise days before the pediatric RIFLE criteria are met.[11,12] Sepsis is recognized as the most common precipitant for AKI in critically ill patients, but sepsis upregulates NGAL expression in serum and urine, thus decreasing the predicting value of NGAL for AKI.[13]

There is a rapidly expanding body of evidence that suggests serum Cys-C to have accuracy and value for the early detection and diagnosis of AKI. Furthermore, in selected populations serum Cys C has shown superior performance to serum creatinine.[9] Cystatin-C is mainly a marker for GFR, with serum concentration rising only after the GFR begins to fall. Particularly in children, the independence from height, gender, age, and skeletal muscles, is advantageous.[14] While there are numerous urinary biomarkers that have been characterized for the early and non-invasive detection of AKI, few have been documented in septic AKI, and the few studies that are available have notable limitations. However, there is an evidence to suggest that selected urinary biomarkers may aid in the early detection of AKI in sepsis and have a predictive value. Early detection of AKI may facilitate the development of new therapies, as the early detection of myocardial ischaemia has done in cardiology. Thus, prospective studies are needed to accurately describe the role and course of these biomarkers in septic AKI and their significance for clinical practice. The RIFLE criteria as the only measure of AKI should be used with caution in clinical settings. A panel of different biomarkers of AKI in plasma and urine might bring the AKI diagnosis forward.

A limitation of this study was its cross-sectional design, in which we took specimens only once, at the time of admission, without considering the duration of illness.

In this study, we find a positively correlated relationship between sCys-C and uNGAL in septic children. Increased sCys-C in septic children was significantly associated with uNGAL. Our findings indicate that serum Cys-C and uNGAL are valuable immediate biomarkers for acute renal injury in pediatric patients with sepsis. The measurement of serum Cys-C provides an efficient diagnostic device to detect the development and characterize the severity of an early deterioration of kidney function initiated by ischemic injury. Additional measurements of urinary NGAL, however, help to assess kidney injury.

Conflict of interest

None declared

References

1. Riedemann NC, Guo RF, Ward PA. The enigma of sepsis. J Clin Invest. 2003;112:460-7.
2. Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS, Wenzel RP. The natural history of the systemic inflammatory response syndrome (SIRS): a prospective study. JAMA. 1995:273;117-23.
3. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatso H, Morgera S, *et al*. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA. 2005:294;813-8.
4. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001:29;1303-10.
5. Edelstein CL and Schrier RW. Pathophysiology of ischemic acute renal failure. In: Schrier RW, editor. Diseases of the kidney and urinary tract. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1041-69.
6. Cowland JB, Borregaard N. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics.

1997:45;17-23.
7. Cowland JB, Sorensen OE, Sehested M, Borregaard N. Neutrophil gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1β but not by TNF-α. J Immunol. 2003;171:6630-9.
8. Mishra J, Mori K, Ma Q, Kelly C, Yang J, Mitsnefes M, *et al*. Amelioration of ischemic acute renal injury by neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol. 2004:15;3073-82.
9. Bagshaw SM, Bellomo R. Cystatin C in acute kidney injury. Curr Opin Crit Care. 2010;16:533-9.
10. Goldstein B, Giroir B, Randolph A, members of International Consensus Conference on Pediatric Sepsis. International pediatrics sepsis concensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med. 2005;6:2-8.
11. Bagshaw S, Bennett M, Haase M, Haase-Fielitz A, Egi M, Morimatsu H, D'amico G, *et al*. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. Intensive Care Med. 2010;36:452–61.
12. Wheeler DS, Devarajan P, Ma Q, Harmon K, Monaco M, Cvijanovich N, Wong HR. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. Crit Care Med. 2008;36:1297–303.
13. Bailey D, Phan V, Litalien C, Ducruet T, Mérouani A, Lacroix J, *et al.* Risk factors of acute renal failure in critically ill children: a prospective descriptive epidemiological study. Pediatr Crit Care Med. 2007;8:29–35.
14. Seitz S, Rauh M, Gloeckler M, Cesnjevar R, Dittrich S, Koch AM. Cystatin C and neutrophil gelatinase-associated lipocalin: biomarkers for acute kidney injury after congenital heart surgery. Swiss Med Wkly. 2013;143:13744.