Journal of Critical Care (2013) **28**, 564–570

ELSEVIER

Journal of Critical Care

New biomarker panel of plasma neutrophil gelatinase–associated lipocalin and endotoxin activity assay for detecting sepsis in acute kidney injury☆

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Keywords:
Acute kidney injury;
Sepsis;
Biomarker;
Endotoxin;
Neutrophil gelatinase–associated lipocalin

Abstract

Purpose: Septic acute kidney injury (AKI) shows an unacceptably high mortality rate. Detection of sepsis is important for the clinical management of AKI patients. This study was undertaken to evaluate 2 biomarkers of neutrophil gelatinase–associated lipocalin (NGAL) and endotoxin activity (EA) assay and their combination for detecting sepsis in AKI.

Materials and Methods: Adult intensive care unit patients consisting of 40 non-AKI, 65 AKI without sepsis, 10 non-AKI with sepsis, and 24 septic AKI were examined in a cross-sectional manner. Plasma NGAL and EA values in whole blood were measured at recruitment. We evaluated whether combining 2 different biomarkers would improve the performance of each biomarker using receiver operating characteristic analysis.

Results: Plasma NGAL was significantly higher in septic AKI patients than in the other AKI patients and non-AKI patients, whereas EA values were higher in septic patients than nonseptic patients irrespective of AKI complication. Combination of plasma NGAL and EA value increased the area under the curve of the receiver operating characteristic curve and showed better performance compared with a clinical model consisting of clinically available variables.

Conclusion: Combinations of plasma NGAL and EA, which are operating via different pathological pathways, significantly improved their detection performance in complicated conditions of septic AKI.

☆ This study was partly supported by Alere Medical Co, Ltd.

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0883-9441/$ – see front matter
http://dx.doi.org/10.1016/j.jcrc.2013.01.009

1. Introduction

Acute kidney injury (AKI) in intensive care units (ICUs) is frequently complicated by sepsis. A multinational prospective observational report described that the most frequent contributing factor to AKI is sepsis, which is seen at a rate of approximately 50% [1]. Other reports have stated that 45% to 70% of all AKIs are associated with sepsis [2-4]. It is also widely recognized that patients with both sepsis and AKI have an unacceptably high mortality rate [2]. Therefore, detection of sepsis in AKI patients is of great importance to enable adequate treatment in these critically ill patients and to improve their outcomes.

Plasma neutrophil gelatinase–associated lipocalin (NGAL) is an emerging biomarker for AKI. Its performance in early detection of renal damage has been valuable in several AKI cohorts [5]. However, plasma NGAL levels would be increased by sepsis. Bagshaw and colleagues [6] reported that plasma NGAL was higher in septic AKI patients than in nonseptic AKI patients. Another report described plasma NGAL was not significantly different between septic shock patients with and without AKI [7]. These results indicate that plasma NGAL might be able to detect sepsis in AKI.

Endotoxin activity (EA) assay, recently approved by the US Food and Drug Administration as an ex vivo diagnostic test of endotoxemia, uses the biological response of patient neutrophil and complement system to an immunological complex of endotoxin and antiendotoxin antibody. It requires only 30 minutes to measure blood endotoxin levels as EA values, which are expressed in relative units derived from the integral of the basal and maximally stimulated (4600 pg/mL lipopolysaccharide) chemiluminescent response [8]. The Multi-Center Endotoxin Detection in Critical Illness (MEDIC) study demonstrated that higher EA levels are associated with a higher risk of mortality as well as an increased risk for developing sepsis [9]. However, it has not been evaluated to date whether the EA assay can detect sepsis complicated with AKI accurately. Neutrophil activation plays a crucial role in AKI pathogenesis [10] and might therefore have a marked impact on EA assay. Therefore, evaluation using EA assay in AKI populations will be necessary before translating this assay to clinical use.

This study was conducted to evaluate whether these 2 biomarkers can profoundly affect detection of sepsis in AKI patients. We also examined the potential of the combination of 2 different biomarkers for sepsis diagnosis in AKI.

2. Materials and methods

2.1. Participants and study design

All patients in this study were older than 20 years and had been admitted to the ICU of The University of Tokyo Hospital. This study enrolled ICU patients in a cross-sectional manner from April 2010 to January 2011. Patients with end-stage renal disease or renal transplant were excluded from these cohorts. Patients with severe neutropenia ($<500/\mu L$) at ICU admission were not enrolled because the EA assay was not expected to measure endotoxin levels in blood correctly. The study protocol was approved by The University of Tokyo Institutional Review Board. Informed consent was obtained from each participant or the participant's family.

The following clinical variables were evaluated: age, sex, blood urea nitrogen (BUN), creatinine, neutrophil count, albumin, lactate, length of ICU stay, and ICU mortality. This information was obtained from medical records. Acute kidney injury was determined by changes in serum creatinine according to the RIFLE criteria at ICU admission. *Acute kidney injury* was defined as a 50% increase from the baseline [11]. *Baseline serum creatinine* was defined as the last outpatient value within 3 months before admission. For a patient with no creatinine measurement within the last 3 months or any known prior creatinine value, the baseline was estimated using the Modification of Diet in Renal Disease equation for the lower end of the reference range (ie, 75 mL/[min 1.73 m^2]) as the RIFLE criteria suggested [11].

Diagnosis of sepsis was made according to the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference Committee guidelines [12]. The severity of illness aside from AKI was assessed according to the nonrenal Sequential Organ Failure Assessment (SOFA) score, from which the renal system score (determined by serum creatinine concentration) was omitted [13,14].

2.2. Biomarker measurement

Biomarker measurement was conducted at enrollment (within 24 hours after ICU admission). Plasma NGAL was determined using the Triage NGAL Device (Alere Medical, Inc, San Diego, CA) as described previously [15]. Briefly, EDTA-anticoagulated whole blood was supplied to the assay device, which contains an NGAL-specific monoclonal antibody conjugated to a fluorescent nanoparticle. The specimen moves through an integrated filter to separate cells from plasma. The plasma then reconstitutes the fluorescent antibody conjugate detection nanoparticles and flows down the diagnostic lane via capillary action. The device is then inserted into the Triage Meter, a portable fluorescence spectrometer. Then quantitative measurements of NGAL concentration are displayed on the meter screen and printout after approximately 15 minutes. The reference range of plasma NGAL is reportedly 60.0 to 78.5 ng/mL [16].

The EA assay is based on the endotoxin reaction with a specific antiendotoxin antibody. Complement proteins, which opsonize the endotoxin-antibody complex, prime neutrophils to enhance respiratory bursts. The activated