AJKD
Original Investigation

Long-term Stability of Urinary Biomarkers of Acute Kidney Injury in Children

Meredith P. Schuh, MD,[1] Edward Nehus, MD,[1] Qing Ma, MD,[1] Christopher Haffner, MS,[1] Michael Bennett, PhD,[1] Catherine D. Krawczeski, MD,[2] and Prasad Devarajan, MD[1]

Background: Recent meta-analyses support the utility of urinary biomarkers for the diagnosis and prognosis of acute kidney injury. It is critical to establish optimal sample handling conditions for short-term processing and long-term urinary storage prior to widespread clinical deployment and meaningful use in prospective clinical trials.

Study Design: Prospective study.

Setting & Participants: 80 children (median age, 1.1 [IQR, 0.5-4.2] years) undergoing cardiac surgery with cardiopulmonary bypass at our center. 50% of patients had acute kidney injury (defined as ≥50% increase in serum creatinine from baseline).

Predictors: We tested the effect on biomarker concentrations of short-term urine storage in ambient, refrigerator, and freezer conditions. We also tested the effects of multiple freeze-thaw cycles, as well as prolonged storage for 5 years.

Outcomes: Urine concentrations of neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), and interleukin 18 (IL-18).

Measurements: All biomarkers were measured using commercially available kits.

Results: All 3 biomarkers were stable in urine stored at 4°C for 24 hours, but showed significant degradation (5.6%-10.1% from baseline) when stored at 25°C. All 3 biomarkers showed only a small although significant decrease in concentration (0.77%-2.9% from baseline) after 3 freeze-thaw cycles. Similarly, all 3 biomarkers displayed only a small but significant decrease in concentration (0.84%-3.2%) after storage for 5 years.

Limitations: Only the 3 most widely studied biomarkers were tested. Protease inhibitors were not evaluated.

Conclusions: Short-term storage of urine samples for measurement of NGAL, KIM-1, and IL-18 may be performed at 4°C for up to 24 hours, but not at room temperature. These urinary biomarkers are stable at −80°C for up to 5 years of storage. Our results are reassuring for the deployment of these assays as biomarkers in clinical practice, as well as in prospective clinical studies requiring long-term urine storage.
Am J Kidney Dis. 67(1):56-61. © *2016 by the National Kidney Foundation, Inc.*

INDEX WORDS: Acute kidney injury (AKI); urinary biomarker; biomarker stability; urine storage; freeze-thaw cycle; sample handling conditions; neutrophil gelatinase-associated lipocalin (NGAL); kidney injury molecule 1 (KIM-1); interleukin 18 (IL-18); enzyme-linked immunosorbent assay (ELISA); acute renal failure; children; pediatric patients.

The use of urinary biomarkers for early detection and prognostication of acute kidney injury (AKI) is a rapidly developing field within nephrology research. In particular, the most promising biomarkers, such as neutrophil gelatinase-associated lipocalin (NGAL),[1] kidney injury molecule 1 (KIM-1),[2] and interleukin 18 (IL-18),[3] have now been investigated to the point that systematic reviews and meta-analyses of their diagnostic utility have recently appeared.[4-7] A number of pivotal prospective clinical studies are now collecting serial urine samples at various points in time for batch analysis of biomarkers at a much later date. In addition, clinical platforms for the measurement of select urinary biomarkers of AKI (eg, NGAL) have been launched.[8] It therefore is critical to establish optimal sample handling conditions for both short- and long-term urinary storage.[9]

In the present study, we investigated the stability of urine NGAL, KIM-1, and IL-18 in a cohort of children undergoing cardiac surgery with cardiopulmonary bypass at our center. We first evaluated the effect on biomarker concentrations of short-term storage over 24 hours in ambient, refrigerator, and freezer conditions. These findings are directly pertinent to obtaining reliable and reproducible results in the clinical setting. We then analyzed the effect of

From the [1]Division of Nephrology, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, OH; and [2]Division of Pediatric Cardiology, Lucile Packard Children's Hospital, Stanford University, Palo Alto, CA.

Received January 22, 2015. Accepted in revised form April 21, 2015. Originally published online May 29, 2015.

Address correspondence to Prasad Devarajan, MD, Cincinnati Children's Hospital Medical Center, MLC 7022, 3333 Burnet Ave, Cincinnati, OH 45229-3039. E-mail: prasad.devarajan@cchmc.org

© *2016 by the National Kidney Foundation, Inc.*

0272-6386
http://dx.doi.org/10.1053/j.ajkd.2015.04.040

multiple freeze-thaw cycles, as well as the stability of these urinary biomarkers after prolonged storage (for 5 years). These results are highly relevant to clinical and translational research studies aimed at biomarker measurements in urine samples following long-term storage.

METHODS

Patient Population

This study was approved by the Institutional Review Board of Cincinnati Children's Hospital Medical Center. All patients younger than 18 years undergoing cardiac surgery with cardiopulmonary bypass at our center from January 2004 through July 2007 were approached for study inclusion. The demographics of the original cohort of 220 patients have been previously described.[10] Percentage increase in serum creatinine level was calculated during the index hospitalization, with AKI defined as a 50% increase in serum creatinine level from baseline. Urine samples for biomarker analysis were obtained at baseline and at various time points after initiation of cardiopulmonary bypass. For this study, we chose urine samples from 80 patients obtained at 2 to 6 hours after bypass because this time point yielded the highest biomarker concentration in previous studies.[10] Samples were chosen based on availability, from patients selected for even sex distribution and other characteristics representative of the original cohort. All urine samples were centrifuged upon collection and the supernatant was divided into 1-mL aliquots and processed in one of the following 3 methods, as illustrated in Fig 1. No protease inhibitors were used.

Processes

Process 1: Effect of Temperature on Short-term Storage

Twenty urine samples were aliquoted and subjected to immediate biomarker testing (baseline, with testing initiated within 15 minutes of aliquoting) or tested after storage for 24 hours at −80°C, 4°C, or 25°C (each on a separate aliquot).

Process 2: Effect of Freeze-Thaw Cycles

The effect of multiple freeze cycles on biomarker stability was evaluated in the same 20 participants as for process 1. After immediate biomarker testing (baseline), samples were tested after a series of 3 sequential freeze-thaw cycles following storage for 24 hours at −80°C.

Process 3: Effect of Long-term Storage

Sixty urine samples were aliquoted and subjected to immediate biomarker testing (baseline) or tested after storage at −80°C for 3 or 5 years (separate aliquots).

Biomarker Measurements

Single measurements of each biomarker were accomplished in a blinded fashion as previously described.[10] The urine NGAL enzyme-linked immunosorbent assay (ELISA) was performed using a commercially available assay (NGAL ELISA Kit 036; Bioporto) that specifically detects human NGAL and uses monoclonal capture and detection antibodies. The starting dilution factor for this assay is 1:500. The lower limit of detection for the NGAL ELISA is 4 pg/mL. Intra- and interassay coefficients of variation were 2.1% and 9.1%, respectively. The urine KIM-1 ELISA was constructed using commercially available reagents (DuoSet DY1750; R&D Systems) as described previously.[11] The KIM-1 ELISA uses affinity-purified polyclonal capture and detection antibodies. The starting dilution for the KIM-1 ELISA is 1:1. The lower limit of detection is 59 pg/mL. Intra- and interassay coefficients of variation were 2.0% and 7.8%, respectively. The urine IL-18 ELISA kit was from Medical and Biological Laboratories. The IL-18 ELISA uses monoclonal capture and detection antibodies. The starting dilution for the IL-18 ELISA is 1:1. The lower limit of detection is 12.5 pg/mL. Intra- and interassay coefficients of variation were 7.5% and 7.3%, respectively.

Statistical Analysis

Biomarker concentrations are reported as median and interquartile range (IQR). Analysis of variance with repeated measures was used to evaluate change in biomarker concentrations during each process. We applied a log transformation (natural log) to biomarker concentrations in our statistical analyses because the data were not normally distributed. Significance was set at $P < 0.05$ after Bonferroni correction for multiple comparisons (6 comparisons for processes 1 and 2 and 3 comparisons for process 3). All statistical analyses were performed using SAS, version 9.3, statistical software (SAS Institute Inc).

RESULTS

Study Participants

Median age of the entire cohort (N = 80) was 1.1 (IQR, 0.5-4.2) years. Twenty unique patients (10 with AKI and 10 without AKI) participated in processes 1 and 2, and 60 additional patients (30 with AKI and 30

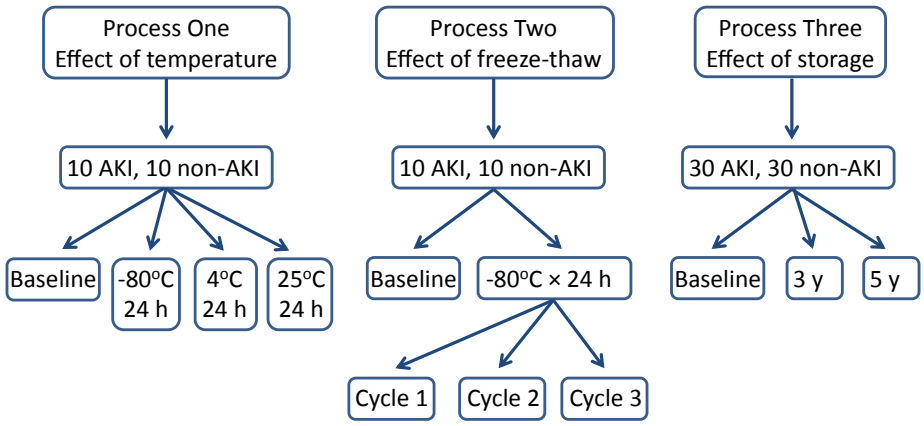

Figure 1. Schematic representation of the study design. Abbreviation: AKI, acute kidney injury.

Download English Version:

https://daneshyari.com/en/article/6156549

Download Persian Version:

https://daneshyari.com/article/6156549

Daneshyari.com