Biomarkers for Sepsis-Associated Acute Kidney Injury

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Rationale

• There are already good AKI biomarkers. Why bother searching for biomarkers for sepsis associated-AKI (SAKI)?
• SAKI results from a complex interplay between inflammation, oxidant injury, and microvascular alterations.
• Biomarkers that perform well for AKI secondary to ischemia, may not perform as well for SAKI.
Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock

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Objective: To validate serum neutrophil gelatinase-associated lipocalin (NGAL) as an early biomarker for acute kidney injury in critically ill children with septic shock.

Design: Observational cohort study.

Setting: Fifteen North American pediatric intensive care units (PICUs).

Patients: A total of 143 critically ill children with systemic inflammatory response syndrome (SIRS) or septic shock and 25 healthy controls.

Interventions: None.

Measurements and Main Results: Serum NGAL was measured during the first 24 hrs of admission to the PICU. Acute kidney injury was defined as a blood urea nitrogen concentration >100 mg/dL, serum creatinine >2 mg/dL in the absence of preexisting renal disease, or the need for dialysis. There was a significant difference in serum NGAL between healthy children (median 80 ng/mL; interquartile ratio [IQR] 55.5–105.5 ng/mL), critically ill children with SIRS (median 107.5 ng/mL; IQR 80–178.5 ng/mL), and critically ill children with septic shock (median 302 ng/mL, IQR 151–570 ng/mL, p < .001). Acute kidney injury developed in 22 of 143 (15.4%) critically ill children. Serum NGAL was significantly increased in critically ill children with acute kidney injury (median 355 ng/mL, IQR 166–1322 ng/mL) compared to those without acute kidney injury (median 186 ng/mL, IQR 98–365 ng/mL, p = .009).

Conclusions: Serum NGAL is a highly sensitive but nonspecific predictor of acute kidney injury in critically ill children with septic shock. Further validation of serum NGAL as a biomarker of acute kidney injury in this population is warranted. (Crit Care Med 2008; 36:1297–1303)

Kr Wons: serum neutrophil gelatinase-associated lipocalin; biomarker; acute kidney injury; septic shock

Acute kidney injury (AKI), formerly known as acute renal failure, is a very common and potentially devastating problem in critically ill children and adults.

A sudden abnormal change in renal function results in a rapid accumulation of metabolic waste products in the blood. These toxins can lead to life-threatening complications and can be a significant contributor to mortality in critically ill children. The reported incidence of AKI in this population varies greatly due to the lack of a standard, consensus definition. For example, AKI affects between 5% and 56% of critically ill patients in reported series (1–5). Unfortunately, the mortality and morbidity associated with AKI remain unacceptably high, up to 60% mortality in critically ill children and adults with multiple organ dysfunction syndrome or MODS. While this dismal prognosis is partly attributable to other comorbid conditions, recent studies have revealed that AKI may be an independent risk factor for mortality in both critically ill children (5–7) and adults (8–11). In addition, the treatment of AKI imposes an enormous financial burden on society, with annual U.S. medical expenditures approaching $8 billion in adults alone (12). Effective treatments to prevent AKI are lacking, and management is largely directed toward reversing the underlying cause (e.g., renal ischemia secondary to hypotension) and providing supportive care. Supportive care in the pediatric intensive care unit (PICU) has traditionally included optimizing fluid status and avoiding potentially nephrotoxic medications, as well as maintaining cardiorespiratory stability with vasoactive medications and mechanical ventilatory support. Renal replacement therapy (RRT) is the only available, proven therapy for critically ill children with AKI, and studies suggest that early initiation of RRT significantly improves survival in children with AKI secondary to septic shock and MODS (13–16).

Sepsis and its related syndromes account for significant morbidity and mortality in critically ill children, accounting for nearly 4,500 deaths and close to $2 billion per year in healthcare expenditures in the United States alone (17). Shock and subsequent multiple organ dysfunction are significant risk factors for mortality in these patients. Sepsis remains a significant risk factor and one of the leading causes of AKI in critically ill children (3–6, 13, 14, 18–21).

In current clinical practice, AKI is typically diagnosed by measuring serum creatinine. However, it is well known that

- NGAL levels greater in children with septic shock, compared to SIRS and normals.
- NGAL levels greater in SAKI compared to no SAKI.
- AUC for SAKI = 0.68
NGAL levels greater in septic AKI compared to non septic AKI.

For discriminating progression to AKI the AUC = 0.71.
Rationale

• Why are we even worried about identifying patients with sepsis who are at risk for AKI?
• More informed implementation of AKI preventive measures.
• More informed decision making regarding initiation of renal replacement therapy.
• Future: more informed decision making surrounding AKI-specific therapies.
Identification of candidate serum biomarkers for severe septic shock-associated kidney injury via microarray


Abstract

Introduction: Septic shock-associated acute kidney injury (SSAki) carries high morbidity in the pediatric population. Effective treatment strategies are lacking, in part due to poor detection and prediction. There is a need to identify novel candidate biomarkers of SSAki. The objective of our study was to determine whether microarray data from children with septic shock could be used to derive a panel of candidate biomarkers for predicting SSAki.

Methods: A retrospective cohort study compared microarray data representing the first 24 hours of admission for 179 children with septic shock with those of 53 age-matched normal controls. SSAki was defined as a $>$200% increase of baseline serum creatinine, persistent to 7 days after admission.

Results: Patients with SSAki ($n = 31$) and patients without SSAki ($n = 148$) were clinically similar, but SSAki carried a higher mortality (45% vs. 10%). Twenty-one unique gene probes were upregulated in SSAki patients versus patients without SSAki. Using leave-one-out cross-validation and class prediction modeling, these probes predicted SSAki with a sensitivity of 98% (95% confidence interval (CI) = 81 to 100) and a specificity of 80% (95% CI = 72 to 86). Serum protein levels of two specific genes showed high sensitivity for predicting SSAki: matrix metalloproteinase-8 (89%, 95% CI = 64 to 98) and elastase-2 (83%, 95% CI = 58 to 96). Both biomarkers carried a negative predictive value of 95%. When applied to a validation cohort, although both biomarkers carried low specificity (matrix metalloproteinase-8: 41%, 95% CI = 28 to 50; and elastase-2: 49%, 95% CI = 36 to 62), they carried high sensitivity (100%, 95% CI = 68 to 100 for both).

Conclusions: Gene probes upregulated in critically ill pediatric patients with septic shock may allow for the identification of novel candidate serum biomarkers for SSAki prediction.

Introduction

Septic shock leads to significant morbidity and mortality in critically ill adult and pediatric patients. Meanwhile, acute kidney injury (AKI) is also known to be independently associated with mortality and morbidity in critically ill patients. The treatment of sepsis costs the US population over $15 billion/year for adults and over $2 billion/year for children, while the costs for AKI approach $10 billion/year for adults alone. Sepsis is the most common precipitant for AKI in both populations, and the development of kidney injury in the context of sepsis is a poor prognostic sign. Together the two disease processes carry up to 75% mortality [5-9].

Effective therapies for septic-shock-associated acute kidney injury (SSAki) are lacking. Detection schemes for SSAki have been and still are dependent on serum creatinine, a flawed real-time marker of AKI [10,11]. Diagnoses of SSAki based upon changes in creatinine, therefore, are considerably varied and create heterogeneity between studies investigating AKI therapy. Biomarker research seeking to identify more robust markers of AKI has yielded promising results. Neutrophil gelatinase-
Approach

- Microarray-based gene expression data from 180 children with septic shock.
- Data represent the first 24 hours of presenting to the ICU with septic shock.
- Whole blood-derived RNA.
- 31 patients with sepsis and AKI (SAKI).
- 149 patients without SAKI.
Statistical Analysis to Derive Candidate Genes for SAKI prediction

• >54,000 gene probes.
• ANOVA with corrections for multiple comparisons.
• False Discovery Rate of 5%.
• SAKI vs. no SAKI.
• 100 gene probes differentially regulated between SAKI and no SAKI.
100 gene probes differentially regulated between SAKI and no SAKI

61 unique and well-annotated genes

21 up-regulated in SAKI, relative to no SAKI

*Candidate SAKI Genes*
Class Prediction Modeling

• Can the expression patterns of the 21 gene probes predict SAKI?
• Leave-one-out cross validation to predict SAKI and no SAKI “classes”. 
### Performance characteristics of class prediction modeling

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<tr>
<th></th>
<th>SAKI</th>
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<tr>
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<td>30</td>
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<tr>
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</table>

- **Sensitivity**: 98% (CI 81 – 100%)
- **Specificity**: 80% (CI 72 – 86%)
- **PPV**: 50% (CI 37 – 63%)
- **+LR**: 4.8 (1.6 – 6.6)
- **NPV**: 99% (CI 95 – 100%)
- **-LR**: 0.04 (0.001 – 0.28)
Can the protein products of these 21 genes serve as biomarkers for SAKI?
Designing an immuno-assay for the candidate SAKI biomarkers

- Limitations
- Small sample volumes
- Magnetic bead multi-plex assay.
- Availability of suitable antibodies.
- Costs.
“Final” biomarker panel

- Elastase 2 (ELA2).
- Fibroblast growth factor 13 (FGF13).
- Matrix metalloproteinase 8 (MMP8).
- Olfactomedin 4 (OLFM4).
- Proteinase 3 (PRTN3).
Biological Plausibility

• Elastase 2 (ELA2).
  – Found in kidney biopsies from patients with autoimmune glomerulonephritis.
• Matrix metalloproteinase 8 (MMP8).
  – Previously reported as a biomarker for renal allograft rejection.
• Proteinase 3 (PRTN3).
  – Auto-antigen for Wegener’s granulomatosis.
• Fibroblast growth factor 13 (FGF13).
  – ?
• Olfactomedin 4 (OLFM4).
  – ?
Deriving a multi-biomarker-based model to predict SAKI

• 200 children with septic shock.
• Measured the 5 candidate biomarkers in serum samples.
• Samples drawn during the first 24 hours of septic shock.
• Classification and Regression Tree (CART) methodology.
CART Analysis

- Classification and Regression Tree.
- Decision tree building technique.
- “Binary recursive partitioning”.
- *Binary*: splitting of patients into 2 groups.
- *Recursive*: can be done multiple times.
- *Partitioning*: entire dataset split into sections.
- Can reveal complex interactions between candidate predictor variables not evident using traditional approaches.
CART Variables

• Primary outcome variable: SAKI on “Day 3” of septic shock.
  – >2 fold increase from baseline creatinine (KDIGO Stage 2 AKI).

• Predictor variables.
  – Biomarker values.
  – Age.
  – Gender.
  – Presence of AKI on “Day 1” of septic shock.
ROOT  N = 200

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MMP8 > 80

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ELA2 ≤ 50

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PRTN3 ≤ 1780

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PRTN3 > 1780

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DAY 1 AKI = NO

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DAY 1 AKI = YES

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OLFM4 > 104

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<tr>
<td>YES</td>
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Low SAKI Risk Terminal Nodes: 0.0% risk
Intermediate SAKI Risk Terminal Nodes: 15 to 22% risk
High SAKI Risk Terminal Nodes: 75% risk
Test Characteristics

- AUC: 0.91 (0.86 – 0.96)
- Sensitivity: 100% (85 – 100)
- Specificity: 62% (54 – 69)
- PPV: 30% (21 – 41)
- NPV: 100% (96 – 100)
Model vs. Day 1 AKI Status Alone

Day 1 AKI alone = 0.83

Biomarker model = 0.91

P = 0.025
Model vs. Day 1 AKI Status Alone

• Net reclassification improvement.
• Does the model add useful information to day 1 AKI status alone?
• Ranges from -2 to +2.
• NRI = 0.96; p < 0.001
Testing the Model

• 200 different patients with septic shock.
• SAKI rate = 10%
• Biomarkers measured and patients classified according to the model with no modifications.
Test Characteristics in the Validation Cohort

• AUC: 0.71 (0.58 – 0.83)
• Sensitivity: 80% (56 – 93)
• Specificity: 51% (44 – 59)
• PPV: 15% (21 – 41)
• NPV: 96% (89 – 99)
Model Calibration

- Combined all 400 subjects from the derivation and validation cohorts.
- Repeated the CART analysis.
Low SAKI Risk Terminal Nodes: 1.4% to 5.8% risk
Intermediate SAKI Risk Terminal Nodes: 15% to 31.4% risk
High SAKI Risk Terminal Nodes: 57% risk
Test Characteristics of the Recalibrated Model

• AUC: 0.84 (0.79 – 0.90)
• Sensitivity: 85% (72 – 93)
• Specificity: 72% (67 – 76)
• PPV: 29% (22 – 38)
• NPV: 97% (94 – 99)
• NRI relative to day 1 AKI status alone: 0.35; p = 0.023
• Outperformed NGAL
Recalibrated model vs. NGAL

NGAL = 0.70
Biomarker model = 0.84
P = 0.003
Summary

• Transcriptomics has enabled the identification of candidate SAKI biomarkers.
• The derived model had excellent performance and contributes information above and beyond day 1 AKI status alone.
• Very modest performance (perhaps poor) in the validation cohort.
• Updated model has test characteristics warranting further exploration.
Work in Progress

• Testing the recalibrated model.
• Develop assays for other candidate genes.
  – 16 candidates were not considered due to technical limitations.
• Combine with other candidate AKI biomarkers.
• Assay the biomarkers in urine as an alternative.
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