Biomarkers of Kidney Injury

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DISCLOSURES: NONE
Objectives

I. Acute Kidney Injury (AKI)
1. Describe the need for biomarkers in AKI
2. Describe the role of biomarkers in AKI
3. Discuss examples of promising AKI biomarkers

II. Renal Dysfunction in Heart Failure (Cardiorenal Syndrome)
1. Describe the prognostic role of old and new biomarkers
2. Discuss the need to stratify patients with “normal” values of traditional markers
3. Discuss the role of new biomarkers in early detection of renal dysfunction
WHAT IS AKI?

- Previously known as acute renal failure (ARF).
- Characterized by an abrupt (hours to days) decline in kidney function. Diagnosis usually based on either an elevation of serum creatinine and/or detection of decreased urine production (oliguria).
- Occurs in a variety of clinical settings. Incidence varies from ~5% of hospitalized patients to >30% of ICU patients, and is increasing dramatically.
- Associated with significantly increased cost of care and substantial morbidity and mortality (e.g. 4 million attributable deaths worldwide per year)
Community-based incidence rates of non-dialysis requiring AKI


> 60% increase from 1996 to 2003
AKI Represents an Independent Risk Factor

Diagnosis of AKI is Often Delayed

- Elevation in serum creatinine is the current gold standard, but this is problematic.
- Normal serum creatinine varies widely with age, gender, diet, muscle mass, muscle metabolism, medications, and hydration status.
- In AKI, serum creatinine can take several days to reach a new steady state.
- Up to 50% of kidney function may be lost before serum creatinine even begins to rise.
RIFLE Criteria for Acute Renal Dysfunction

- **Risk**
  - Increased creatinine x 1.5 or GFR decrease > 50%

- **Injury**
  - Increased creatinine x 2 or GFR decrease > 50%

- **Failure**
  - Increase creatinine x 3 or GFR dec > 75% or creatinine ≥4mg/dl (Acute rise of ≥0.5 mg/dl)

- **Loss**
  - Persistent ARF** = complete loss of renal function > 4 weeks

- **ESRD**
  - End Stage Renal Disease

- **Urine Output Criteria**
  - UO < .5ml/kg/h x 6 hr
  - UO < .5ml/kg/h x 12 hr
  - UO < .3ml/kg/h x 24 hr or Anuria x 12 hrs

**High Sensitivity**

**High Specificity**
Early detection saves lives

## Biomarkers: AMI versus AKI

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Multiple Therapies 50% ↓ Mortality
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- **Multiple Therapies**
  - 50% ↓ Mortality
- **Supportive Care**
  - High Mortality

Need early biomarkers of AKI for improved understanding, early treatment and better outcomes.
The clinical clock is always late

we can identify different milestones along the timeline of AKI. Injury begins inducing molecular modifications subsequently evolving into cellular damage. Cells start to produce biomarkers of injury and only later does the clinical picture of the syndrome develop with the typical sign and symptoms.
Utilities of biomarkers in AKI

- Early diagnosis
- Define severity of injury, monitor AKI course
- Define AKI subtypes & etiology (pre-renal, septic, nephrotoxic)
- Monitor response to AKI interventions
- Risk stratify for poor outcomes (dialysis need, CKD, mortality)
- Identify location of renal tubular injury

Devarajan & Williams, Seminars in Nephrol, 2007
What is an ideal biomarker?

Qualities

- Accurate, reliable
- Relatively non-invasive/acceptable to patients
- Rapidly measurable, standardized assay
- Sensitive/specific with reproducible cutoff values

Nguyen & Devarajan, Ped Nephrol, 2008
Human AKI Biomarkers Currently Under Investigation

- NGAL (urine and plasma)
- KIM-1 (urine)
- L-FABP (urine)
- IL-18 (urine)
- Cystatin C (urine and plasma)
- GST $\alpha$ and $\pi$ (urine)
- GGTP (urine)
- Beta 2-microglobulin (urine)

- Novel Validating
- Established Retesting
Relation of GFR changes with serum creatinine and cystatin C levels

Cystatin C, compared to Creatinine, is a more reliable, early marker of renal dysfunction.

Cystatin C concentration is not influenced by inflammation, muscle mass, gender, or age.

Artunc F et al, J Cardiol 2005;102; 173
The superiority of Cystatin C- over Creatinine-based GFR Assessments in detection of early GFR decrease

ROC plots for the diagnostic accuracy of serum Cystatin C and serum Creatinine to detect GFR < 78 mL/min/1.73 m²

Stabuc B et al., Clin Chem 2000; 46; 193
What is NGAL?

- Neutrophil gelatinase-associated lipocalin (NGAL)
  - First described as a 25 kDa protein bound to gelatinase from neutrophils
  - Also known as lipocalin-2 and siderocalin.
  - Known to play a role in fighting bacteria infections

- Animal studies have shown NGAL is one of the earliest proteins induced in the kidney after ischemic or nephrotoxic insult.

- Expanded studies have shown urinary NGAL to be an early marker of AKI in a variety of settings.

Devarajan, Expert Opin Med Diag 2008
Kidney NGAL in Experimental Ischemic AKI

- Mouse Ischemia
- 30 min ischemia
- S creat ↑ 24 h
- Kidney NGAL ↑ 3 h
- Colocalize with PCNA (proliferating cell nuclear antigen)

Mishra et al, JASN 15:3073-82, 2004
Urine NGAL in Experimental Ischemic AKI

- Mouse Ischemia
- 30 min ischemia
- S creat ↑ 24 h
- Urine NAG ↑ 8 h
- Urine β2M ↑ 8 h
- Urine NGAL ↑ 2 h

Mishra et al, JASN 15:3073-82, 2004
Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery

Jaya Mishra*, Catherine Dent*, Ridwan Tarabishi*, Mark M Mitsnefes, Qing Ma, Caitlin Kelly, Stacey M Ruff, Kamyar Zahedi, Mingyuan Shao, Judy Bean, Kiyoshi Mori, Jonathan Barasch, Prasad Devarajan
Detection of Urinary NGAL by ELISA

**Urine NGAL is upregulated 15-fold within 2 hours after CPB in patients who later develop ARF**

# Urine and serum NGAL test characteristics

Adapted from: Mishra, et al. Lancet 2005

<table>
<thead>
<tr>
<th>Cutoffs for 2-h urine NGAL</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
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<tr>
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<td>80 µg/L</td>
<td>0.90</td>
<td>1.00</td>
<td>1.00</td>
<td>0.96</td>
</tr>
<tr>
<td>100 µg/L</td>
<td>0.70</td>
<td>1.00</td>
<td>1.00</td>
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<th>Cutoffs for 4-h urine NGAL</th>
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<td>100 µg/L</td>
<td>0.65</td>
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<td>0.89</td>
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<td>50 µg/L</td>
<td>0.50</td>
<td>1.00</td>
<td>1.00</td>
<td>0.84</td>
</tr>
<tr>
<td>80 µg/L</td>
<td>0.20</td>
<td>1.00</td>
<td>1.00</td>
<td>0.76</td>
</tr>
</tbody>
</table>

AUC at 2 hours post surgery: Urine = 0.998  Serum = 0.906
NGAL as an Early AKI Biomarker

<table>
<thead>
<tr>
<th>Biomarker Name</th>
<th>Cardiopulmonary Bypass (CPB)</th>
<th>Contrast induced Nephropathy</th>
<th>Sepsis or ICU Setting</th>
<th>Kidney Transplant (tx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGAL (ROC AUC)</td>
<td>2 hr post CPB 2 days pre AKI 0.91-0.99 (1-5)</td>
<td>2 hr post contrast 1-2 days pre AKI 0.92 (6, 7)</td>
<td>2 days pre AKI 0.78 (8, 9)</td>
<td>12 hr post tx 2-3 days pre DGF 0.90 (10, 11)</td>
</tr>
</tbody>
</table>

AKI = 50% or greater increase in serum creatinine from baseline
DGF = dialysis requirement within the first week after transplant

(2) Dent et al, Crit Care 2007, 11(6):R127 (P)
(3) Bennett et al, CJASN 2008, 3:665-73 (U)
(4) Portilla et al, KI 2008, 4:465-72 (U)
(5) Wagener et al: Anesthesiol 2006, 105: 485-491 (U; AUC 0.78)
(7) Bachorzewska-Gajewska et al, NDT 2007, 22:295-6 (U+P)
(8) Zappitelli et al, Crit Care 2007, 11(4):R84 (U)
(10) Parikh et al, Am J Transplant 2006, 6:1639-45 (U)
(11) Kusaka et al, Cell Transplant 2008, 17:1-6 (P)

U=Urine  P=Plasma

Thomas L. Nickolas, MD, MS; Matthew J. O’Rourke, BS; Jun Yang, MD, PhD; Meghan E. Sise, BS; Pietro A. Canetta, MD; Nicholas Barasch, BS; Charles Buchen; Farris Khan, MD; Kiyoshi Moti, MD, PhD; James Giglio, MD; Prasad Devarajan, MD; and Jonathan Barasch, MD, PhD

Presenting serum creatinine by diagnostic group

Urine NGAL by diagnostic group

Biomarkers and clinical outcomes

Table 3. Multivariate Analysis of Acute Kidney Injury Biomarker Levels Derived from Receiver-Operating Characteristic Curve Analysis*

<table>
<thead>
<tr>
<th>Model Parameters</th>
<th>Odds Ratio (95% CI)</th>
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<tbody>
<tr>
<td>NGAL level (&gt;130 μg/g)</td>
<td>24.70 (7.69–79.42)</td>
</tr>
<tr>
<td>α₁-Microglobulin level (&gt;35 mg/g)</td>
<td>1.85 (0.80–4.31)</td>
</tr>
<tr>
<td>α₁-Acid glycoprotein level (&gt;21 mg/g)</td>
<td>0.741 (0.33–1.69)</td>
</tr>
<tr>
<td>NAG level (&gt;4.5 U/g)</td>
<td>1.07 (0.52–2.18)</td>
</tr>
<tr>
<td>Presenting creatinine level &gt;221 μmol/L (&gt;2.5 mg/dL)</td>
<td>6.03 (2.25–16.14)</td>
</tr>
<tr>
<td>Blood urea nitrogen level (per mg/dL increase)</td>
<td>1.01 (1.00–1.03)</td>
</tr>
<tr>
<td>Serum leukocyte count (per cells × 10⁹/L increase)</td>
<td>1.05 (1.01–1.10)</td>
</tr>
</tbody>
</table>

* Regression analysis of biomarkers as predictors of combined clinical outcomes (nephrology consultation, intensive care unit admission, dialysis initiation, or mortality). NAG = N-acetyl-β-D-glucosaminidase; NGAL = neutrophil gelatinase-associated lipocalin.
The role of Kidney Injury Molecule-1 (KIM-1) in detection of AKI

Han et al. Kidney International 2003
Critical Illness: unknown timing of AKI

Critically ill adults: retrospective. Landmark study.

SCr rise
IL-18

Figure 2. Receiver operating characteristic (ROC) curve and performance characteristics for urine IL-18 measured 1 d before AKI. The area under the ROC curve for the urine IL-18 test is 73%, demonstrating a good performance for the diagnosis of AKI within the next 24 h.

Parikh et al, JASN, 2005
Urinary Panel for Early Diagnosis of AKI after temporally defined events

In analogy with cardiac markers Modified from P. Devarajan
Plasma Panel for Early Diagnosis of AKI after temporally defined events

In analogy with markers of myocardial injury  Modified from P. Devarajan
Mechanisms of Acute Kidney Injury in Cardiac Disease

Ronco et al. JACC 2008;52:1528
Prognostic Value of BUN in Patients Hospitalized With Worsening Heart Failure: (ACTIV in CHF sub-study)

Baseline Renal Dysfunction and Worsening Renal Function (WRF) are Additive in Predicting Mortality in HF Patients

Predictors of WRF were thiazide diuretics, increased BUN, and vascular disease.

And a fall in sCr of >0.3 mg/dL was associated with improved mortality.

<table>
<thead>
<tr>
<th>sCreatinine</th>
<th>WRF (≥0.3mg/dL)</th>
<th>≤1.2</th>
<th>1.2-2.0</th>
<th>≥2.0</th>
<th>≤1.2</th>
<th>1.2-2.0</th>
<th>≥2.0</th>
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<tr>
<td></td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
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Prognostic value of Cystatin-C in AHF patients with normal creatinine

Mortality at 1 year 12.6 vs. 40.4%  \( P < 0.0001 \)

Combining cystatin C and NT-proBNP levels for risk stratification in AHF patients with renal dysfunction

Relation of Cystatin C with Diastolic and RV Function in HF

A

Spearman's $r = 0.34$, $p < 0.0001$

B

RV systolic dysfunction class

- Rank sums $p = 0.003$
- 0 (n=45)
- 1-2 (n=58)
- 3-4 (n=35)

C

MR severity

- Rank sums $p = 0.008$
- 0 (n=84)
- 1-2 (n=31)
- 3-4 (n=22)

Tang et al. J Card Fail 2009;14:394
Cystatin C and Co-morbidities

- CKD: increased - plasma levels similar to AKI
- Hormonal: increased with high dose steroids and in hyperthyroidism
- Diabetes: increased – in diabetics 64-100 yrs of age, prevalence of nephropathy estimated from Cystatin C is 65%, compared to 21% if serum creatinine is used
- Aging: 0.05 mg/L increase every 10 years – corresponds to a GFR drop of ≈ 6 ml/min

Urinary NGAL, a marker of tubular damage, is increased in patients with CHF

Damman et al. European J Heart Fail 2008;10:997
Plasma NGAL in Predicting Worsening Renal Function in ADHF

Aghel et al. J Cardiac Fail 2010;16:49-54
Prognostic value of NGAL in post-MI HF

Yndestad A et al, EHJ 2009;30:1229
Future Directions

- Conduct large multicenter trials to determine:
  - Effect of co-morbid conditions and confounding factors on AKI biomarker measurements
  - Accuracy and thresholds of biomarkers to diagnose AKI in the presence of confounding variables
  - Utility of AKI biomarkers independent of serum creatinine:
    - “false negatives” in pre-renal AKI
    - “false positives” in intrinsic but sub-clinical AKI
    - Biomarkers for hard clinical outcomes
    - Biomarkers to trigger clinical interventions
Prognostic value of multi-marker approach in AHF: including biomarkers of kidney injury

Manzano et al. Am J Cardiol 2009;103:1753
Biomarkers of Kidney Injury: Take Home Messages

- Acute kidney injury (AKI) is a common and serious problem in routine clinical practice.
- The diagnosis of AKI based on serum creatinine is often delayed.
- In the past, preventive and therapeutic measures were also delayed due to lack of early biomarkers.
- Novel biomarkers (NGAL, Cystatin C, KIM-1, IL-18) may be providing tools for the early prediction of AKI and outcomes, and for testing therapies.
- In ADHF, biomarkers such as Cystatin C and NGAL may predict worsening renal function as well as adverse clinical outcomes in patients with normal creatinine levels.
- More prospective trials are needed in order to clarify the utility of new biomarkers of kidney injury in acute cardiac care.