Application of Novel Biomarkers in Peri-Operative AKI

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Disclosures

– Co-inventor on IL-18 patent issued to University of Colorado (no commercial value)

– NIH grants on Biomarker Discovery & Validation
Outline of the Talk

• Why do we need biomarkers in peri-operative acute kidney injury (AKI)?

• Biomarkers of AKI
  – Biomarker Results from Multicenter studies
  – The future: Application of New Biomarkers
Human Studies

Dialysis
Failure with Therapeutic Development in Kidney Diseases

• Dependence on serum creatinine

  – As enrollment criteria for treatment trials of AKI
    • Miss the “window of opportunity” due to the delayed rise in serum creatinine
    • Cannot differentiate “pre-renal azotemia” from “true kidney injury” and thus the true effect size of the drug is underestimated

  – As an outcome criteria for prevention trials of AKI
    • Not sensitive to small improvements in kidney function
    • “Noise” in the endpoint requiring larger sample size
Biomarkers in Relation to Site of Injury in Nephron

**Proximal Tubule Injury**
- Urine IL-18
- Urine KIM-1
- Urine L-FABP

**Glomerular Filtration and Proximal Tubule Function**
- Serum Creatinine
- Serum Cystatin C
- Urine Cystatin C

**Distal Tubule**
- Urine NGAL

**Glomerular Injury**
- Urine albumin excretion

**Loop of Henle Injury**
- Uromodulin

**Interstitial Fibrosis**
- Urine TGF-β₁

**Biomarkers**
- Glomerular injury
- Proximal tubule function
- Distal tubule
- Glomerular filtration
- Loop of Henle injury
- Interstitial fibrosis
HOW CAN KIDNEY INJURY BIOMARKERS HELP?

• Early detection of AKI
• Predicting outcomes in hospital: Severity of AKI, dialysis, mortality, resource utilization
• Differential diagnosis of AKI
  – Pre-renal, ATN, AIN
• Ascertaining site and etiology of renal injury
• Monitoring effects of an intervention
  – Nephrotoxicity
<table>
<thead>
<tr>
<th>Phase</th>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preclinical Exploratory</td>
<td>Phase 1</td>
<td>Promising directions identified</td>
</tr>
<tr>
<td>Clinical Assay and Validation</td>
<td>Phase 2</td>
<td>Clinical assay detects established disease</td>
</tr>
<tr>
<td>Retrospective Longitudinal</td>
<td>Phase 3</td>
<td>Biomarker detects disease early before it becomes clinically obvious</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Determine sensitivity/specificity</td>
</tr>
<tr>
<td>Prospective Screening</td>
<td>Phase 4</td>
<td>Use biomarker to screen population</td>
</tr>
<tr>
<td></td>
<td></td>
<td>“False referral” rate are identified</td>
</tr>
<tr>
<td>Disease Control</td>
<td>Phase 5</td>
<td>Impact of screening on reducing the burden of disease</td>
</tr>
</tbody>
</table>

Pepe MS et al: Journal of National Cancer Institute, 2001
TRIBE-AKI Consortium

TRANSLATIONAL RESEARCH
INVESTIGATING BIOMARKER
ENDPOINTS- ACUTE KIDNEY INJURY

• Created in 2005
• www.yale.edu\tribeaki
Eligible Patients:
CABG +/- Valve Surgery patients at risk for developing Acute Kidney Injury (AKI)

- Baseline serum creatinine > 2 mg/dL
- Ejection fraction < 35%
- Age > 65 years
- Diabetes mellitus
- Concomitant CABG & valve surgery
- Repeat revascularization surgery

One pre-op blood sample (10mL)
One pre-op urine sample (10mL)

Post-OP Blood Collection
Day 1: 10mL sample
Day 2: 10mL sample
Day 3: 10mL sample
Day 4: 10mL sample
Day 5: 10mL sample

Post-OP Urine Collection
Day 1: 10mL samples X 4
Day 2: 1 10mL sample
Day 3: 1 10mL sample
Day 4: 1 10mL sample
Day 5: 1 10mL sample

Data Collection

## Patient outcomes

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>N=1219</td>
<td></td>
</tr>
<tr>
<td>Severe AKI, n (%)</td>
<td>60  (5%)</td>
</tr>
<tr>
<td>First Day of AKI, median (IQR)</td>
<td>3 (2-4)</td>
</tr>
<tr>
<td>Dialysis, n (%)</td>
<td>18  (1.5%)</td>
</tr>
<tr>
<td>Death, n (%)</td>
<td>20  (1.6%)</td>
</tr>
<tr>
<td>Length of stay, mean (SD)</td>
<td></td>
</tr>
<tr>
<td>In ICU</td>
<td>3.4  (8)</td>
</tr>
<tr>
<td>In hospital</td>
<td>8.5  (10)</td>
</tr>
</tbody>
</table>
Opportunities of Assessing Risk of AKI in Cardiac Surgery

1. Plasma Cystatin C
2. Plasma BNP
3. Urine Albumin

Koyner J, Parikh CR: CJASN 2013
Biomarker results
- Peaked within 6 hours after surgery
- Significantly different between cases and controls
- Declined after peak
Urine NGAL and Plasma NGAL

- Peaked 6 hours after surgery
- Plasma NGAL remains elevated after peak

Parikh CR et al, JASN 2011
Urine KIM-1

- Peaked 2 days after surgery

- Significantly different between cases and controls except 1 day after surgery

Parikh CR et al: CJASN 2013
Urine L-FABP and Urine Cystatin C

Urine L-FABP (ng/mL)

Urine Cystatin C (mg/L)

AKI
Non-AKI
Day of first evidence of AKI (IQR)*
Simplified Framework for Biomarker Analysis & Interpretation

New Biomarker and Outcome

ROC Analysis

AUC>90%

Yes

Good Classifier

No

Not A Good Classifier

Multivariate Association (OR/RR)

Clinically and Statistically Significant

No

Biomarker Not Helpful

Yes

Check incremental value on existing clinical models

NRI/IDI
Change in AUC/C-Index
Urine IL-18 0.74 (0.04)
Urine KIM-1 0.71 (0.04)
Plasma NGAL 0.70 (0.04)
2-way combo 0.76 (0.04)
3-way combo 0.78 (0.04)

Moderate Classifiers
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>AUC (SE)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mild AKI</td>
<td>Severe AKI</td>
</tr>
<tr>
<td>Urine IL-18</td>
<td>0.65</td>
<td>0.74</td>
<td>0.79</td>
</tr>
<tr>
<td>Urine NGAL</td>
<td>0.61</td>
<td>0.67</td>
<td>0.78</td>
</tr>
<tr>
<td>Plasma NGAL</td>
<td>0.67</td>
<td>0.7</td>
<td>0.82</td>
</tr>
<tr>
<td>Urine KIM-1</td>
<td>0.64</td>
<td>0.71</td>
<td>0.75</td>
</tr>
<tr>
<td>Urine L-FABP</td>
<td>0.60</td>
<td>0.61</td>
<td>0.66</td>
</tr>
<tr>
<td>Urine Cystatin C</td>
<td>0.67</td>
<td>0.72</td>
<td>0.70</td>
</tr>
</tbody>
</table>
Prerenal Azotemia Attenuates the Performance of AKI Biomarkers

Parikh CR, Han G: AJKD 2013 (in press)
First post-operative sample and risk of AKI

![Bar chart showing AKI biomarker quintiles for Urine IL-18, Urine NGAL, Plasma NGAL, Urine KIM-1, and Urine L-FABP.](chart.png)
Significant association between biomarkers and AKI

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Unadjusted Odds Ratio (95% CI)</th>
<th>Adjusted Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine IL-18</td>
<td>10.3 (3.3, 46)</td>
<td>6.8 (1.9, 24.3)</td>
</tr>
<tr>
<td>Urine NGAL</td>
<td>4.7 (1.9, 11.7)</td>
<td>2.5 (0.9, 6.8)</td>
</tr>
<tr>
<td>Plasma NGAL</td>
<td>7.8 (2.7, 22.6)</td>
<td>5.0 (1.6, 15.3)</td>
</tr>
<tr>
<td>Urine KIM-1</td>
<td>6.2 (2.1, 18.7)</td>
<td>4.8 (1.6, 14.6)</td>
</tr>
<tr>
<td>Urine L-FABP</td>
<td>2.9 (1.2, 7.1)</td>
<td>1.8 (0.7, 4.6)</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, race, CPB time, non-elective surgery, pre-op eGFR, diabetes, and hypertension
Biomarkers (like AKI) are associated with length of stay

- **Urine IL-18 (pg/mL)**
  
  - Q1: 2
  - Q2: 4
  - Q3: 6
  - Q4: 8
  - Q5: 10

- **Urine NGAL (ng/mL)**
  
  - Q1: 2
  - Q2: 4
  - Q3: 6
  - Q4: 8
  - Q5: 10

- **Plasma NGAL (ng/mL)**
  
  - Q1: 2
  - Q2: 4
  - Q3: 6
  - Q4: 8
  - Q5: 10

Legend:
- Blue: Length of stay in ICU
- Red: Length of stay in hospital
Opportunities of Assessing Risk of AKI in Cardiac Surgery

Pre-operative Assessment
1. Plasma Cystatin C
2. Plasma BNP
3. Urine Albumin

Immediately after Surgery

At AKI Diagnosis

Koyner J, Parikh CR: CJASN 2013
Using Biomarkers To Predict Prognosis on day of AKI
AKI progression was defined as worsening in AKIN Stage (n=45, 11.8%)
## Association of Biomarkers with AKI Progression

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine Albumin to Creatinine Ratio</td>
<td>4.8 (1.9, 11.9)</td>
<td>4.0 (1.5, 10.4)</td>
</tr>
<tr>
<td>Urine NGAL</td>
<td>2.3 (1.1, 4.8)</td>
<td>2.1 (0.9, 4.7)</td>
</tr>
<tr>
<td>Urine IL-18</td>
<td>3.6 (1.6, 8.0)</td>
<td>3.2 (1.4, 7.6)</td>
</tr>
<tr>
<td>Plasma NGAL</td>
<td>11.7 (4.5, 30.3)</td>
<td>9.7 (3.4, 27.5)</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, white race, CPB time > 120 minutes, non-elective surgery, pre-op eGFR (<30, 30-60, >60), diabetes, hypertension, and percent change in post-operative serum creatinine from baseline at the time of AKI diagnosis.

Further multicenter validation
Confirming the predictive value of IL-18

IL-18 was the best predictor of the primary outcome of worsening AKI to AKIN stage 2/3 or death (AUC=0.74) and the secondary outcome of AKIN stage 3 or death (AUC=0.89).

HOW CAN KIDNEY INJURY BIOMARKERS HELP?

- Early detection of AKI
- Predicting outcomes in hospital: Severity of AKI, dialysis, mortality, resource utilization
- Differential diagnosis of AKI
  - Pre-renal, ATN, AIN
- Ascertaining site and etiology of renal injury
- Monitoring effects of an intervention
  - Nephrotoxicity
Adjudication of AKI: The TRIBE AKI Experience
How we did it

• 67 consecutive cases of mild AKI were adjudicated
• Panel of 3 experienced nephrologists
• Independent review of the Case Report Form
  • No biomarker data
• Completed a AKI Checklist (next slide)
  • ATN, Pre-renal, Indeterminate
• Met in person to finalize in the setting of disagreement
<table>
<thead>
<tr>
<th>Category (Circle One)</th>
<th>Description (Check all that apply)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-renal Azotemia</td>
<td>Pattern and kinetics of blood creatinine’s rise and fall during AKI episode.</td>
</tr>
<tr>
<td></td>
<td>Negative fluid balance</td>
</tr>
<tr>
<td></td>
<td>Other: Please print a description:</td>
</tr>
<tr>
<td>Acute Tubular Necrosis (ATN)</td>
<td>Ischemic</td>
</tr>
<tr>
<td></td>
<td>Hypotension and its management around the time of the AKI episode</td>
</tr>
<tr>
<td></td>
<td>CPB time</td>
</tr>
<tr>
<td></td>
<td>Other: Please print a description:</td>
</tr>
<tr>
<td></td>
<td>Nephrotoxic</td>
</tr>
<tr>
<td></td>
<td>Use of contrast agent</td>
</tr>
<tr>
<td></td>
<td>Use of NSAID</td>
</tr>
<tr>
<td></td>
<td>Other: Please print a description:</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Please provide reason(s):</td>
</tr>
</tbody>
</table>


## Breakdown of the Diagnoses

<table>
<thead>
<tr>
<th>Panelists Adjudications</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 ATN</td>
<td>11 (16.4)</td>
</tr>
<tr>
<td>3 PRA</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>3 Indeterminate</td>
<td>0</td>
</tr>
<tr>
<td>2 ATN, 1 PRA</td>
<td>17 (25.3)</td>
</tr>
<tr>
<td>2 ATN, 1 Indeterminate</td>
<td>10 (14.9)</td>
</tr>
<tr>
<td>2 PRA, 1 ATN</td>
<td>4 (6.0)</td>
</tr>
<tr>
<td>2 PRA, 1 Indeterminate</td>
<td>6 (9.0)</td>
</tr>
<tr>
<td>2 Indeterminate, 1 ATN</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>2 Indeterminate, 1 PRA</td>
<td>5 (7.5)</td>
</tr>
<tr>
<td>1 ATN, 1 PRA, 1 Indeterminate</td>
<td>10 (14.9)</td>
</tr>
</tbody>
</table>

- **All 3 Agreed on 13 (~20%)**
- **2 of 3 agreed on 44 (~65%)**
- **0 of 3 agreed on 10 (~15%)**

- **ATN in 41 (~60%)**
- **PRA in 13 (~20%)**
- **Indeterm. in 13 (~20%)**
Adjudication Experience

- ATN is cause of AKI 60% of the time
- All 3 Adjudicators only agreed 20% of the time
- No urinary biomarker predicted ATN
- Peri-operative AKI is a heterogeneous disease where functional and structural injury may coexist
Use Biomarkers to Improve Clinical Trial Design
Biomarkers in AKI trials

1. Biomarkers as entry criteria
   - High risk trial defined by biomarker risk score

2. Biomarkers as surrogate outcomes
   - Acute kidney injury defined by biomarkers
Entry Criteria

Patients undergoing Cardiac Surgery

- Measure biomarkers
- High biomarkers
  - Randomize
  - Intervention
  - Standard of Care
  - Low biomarkers
    - No enrollment
Biomarkers as Surrogates

Randomize

Standard of Care

Cardiac Surgery

Intervention

Day 1

Measure Biomarkers 0-6 Hours

AKI > 0.3mg/dL

In-Hospital Stay

AKI > 100% or dialysis
Summary

• Urine and Plasma biomarkers of AKI are elevated before serum creatinine

• Biomarkers by themselves had only modest classification potential for diagnosis of AKI

• First post-operative values of urine IL-18, urine KIM-1 and Plasma NGAL are independently associated with severe AKI in adults and predict other in-hospital outcomes

• Biomarkers also predict progression of AKI at the time of clinical AKI
Challenges for AKI biomarkers

• Gold standard is serum creatinine

• Cannot separate ATN from prerenal azotemia within AKI participants
  – Peri-operative AKI may be a heterogenous state with co-existence of functional and structural injury

• Need to power studies looking at hard endpoints (long term outcomes)

• Need to use biomarkers in clinical trials of AKI
The TRIBE Team

Co-Investigators
Dr. Steven Coca
Dr. Prasad Devarajan
Dr. Amit Garg
Dr. Jay Koyner
Dr. Catherine Krawczeski
Dr. Simon Li
Dr. Cary Passik
Dr. Uptal Patel
Dr. Michael Shlipak
Dr. Michael Zappitelli

Statisticians
Heather Thiessen-Philbrook

Coordinators:
Isabel Butrymowicz
Rowena Kemp

2nd Annual TRIBE-AKI Consortium Meeting
New Horizon or False Dawn?

Further explorations are needed?
Questions??
HOW CAN KIDNEY INJURY BIOMARKERS HELP?

• Early detection of AKI
• Predicting outcomes in hospital: Severity of AKI, dialysis, mortality, resource utilization
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