Overview of Current Biomarkers in Kidney Disease

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Disclosure: JVB is co-inventor on KIM-1 patents that have been licensed to a number of companies including Johnson and Johnson, Genzyme, R and D, BioAssay Works, and BiogenIdec
Outline

• AKI Biomarkers
• Lessons Learned from Success in Identification and Qualification of Kidney Biomarkers to Guide Drug Development
• The Gold Standard Problem
• Clinical studies of Biomarkers in AKI
• Challenges with CKD Biomarkers
• Biomarkers provide insight into pathophysiology
Characteristics of an Ideal Biomarker for AKI

- Easy to measure in easily obtainable fluids: urine or blood
- Sensitive enough to pick up meaningful injury prior to functional changes
- Produced by a specific segment of the kidney tubule or kidney endothelium
- Distinguishes tubular from prerenal and glomerular injury
- Stable in the blood or urine, devoid of interferences with other substances, and unaffected by chemical composition of the urine, including ionic strength and pH among others
- Increases in levels are predictive of severity of acute injury and chronic sequelae
- Decreases in levels are reflective of recovery
- Understandable function of the marker in the kidney
**Damage Normal Epithelium**

**Toxic/ischemic Injury**

**Apoptosis**

**Necrosis**

**Cell death**

**Death**

**Complications**

**Increased risk**

**Normal**

**Potential biomarkers for early diagnosis of AKI**
- Kidney Injury Molecule-1 (KIM-1)
- Neutrophil Gelatinase Associated Lipocalin (NGAL)
- N-acetyl-β-D-glucosaminidase (NAG)
- Cystatin C
- Interleukin-18 (I-18), microalbuminuria
- FABP, β2 microglobulin, α1-microglobulin, others

**AKIN Scheme, 2006**

**GFR ↓**

**Kidney failure**

**Serum Creatinine**

**Blood Urea Nitrogen**

**Delayed biomarkers for kidney injury**
Candidate Biomarkers for Kidney Injury

Bonventre et al. Nature Biotech 2010
“Additional biomarkers (quantitative measures of biological effects that provide informative links between mechanism of action and clinical effectiveness) and additional surrogate markers (quantitative measures that can predict effectiveness) are needed to guide product development.”
Sensitivity and Specificity of Kim-1, NAG, BUN, SCr

Predictive Safety Testing Consortium Rat Toxicity Studies

Vaidya et al. Nature Biotechnology, May, 2010
FDA and European Medicines Agency Will Consider New Biomarker Test Results When Assessing Kidney Toxicity of Experimental Drugs

In the first use of a framework allowing submission of a single application to the 2 agencies, the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) will allow drug companies to submit the results of 7 new tests that evaluate kidney toxicity…,(FDA news release, 6/17/2008).

The tests measure levels of 7 key biomarker proteins found in urine that can provide information about drug-induced damage to kidney cells (renal toxicity). The new biomarkers are:

- KIM-1
- Albumin
- Clusterin
- Trefoil factor-3
- Cystatin C
- Total protein
- Beta-2 microglobulin


http://www.hivandhepatitis.com/recent/2008/061708_e.html
European InnoMed Predictive Toxicology Consortium ROC curves for urinary biomarkers compared to traditional clinical chemistry parameters.

(b) Urinary kidney biomarkers

- urinary Kim-1: AUC = 0.99, p-value < 0.0001
- urinary clusterin: AUC = 0.93, p-value < 0.0001
- urinary lipocalin-2: AUC = 0.77, p-value = 0.0044
- urinary Timp-1: AUC = 0.79, p-value = 0.0027
- blood urea nitrogen: AUC = 0.64, p-value = 0.132
- serum creatinine: AUC = 0.54, p-value = 0.6513

Flow chart explaining the proposed limited clinical translational use of the new renal biomarkers

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**Pre-clinical**

- Tubular toxicity confirmed by histopathology in one or several species including rat.
- BUN and SCr levels in control range
- Measure BUN, SCr and Kim-1, Albumin in urine samples of a follow-up rat GLP study demonstrating reversibility, interim urine samplings, and periodic histopathologic assessments

- Yes
  - Kim-1, Albumin diagnostic?*

- No
  - Non-monitorable kidney toxicity: Clinical trial delayed unless mechanistic understanding can be developed to address human irrelevance

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**Clinical**

- Phase I / II clinical trial: Monitor Kim-1, Albumin, BUN, SCr. Base decisions on best pre-clinical marker among Kim-1, Albumin, **

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* Sponsor can voluntary measure Albumin or Kim-1 alone or both markers together

** Pre-clinical best marker means marker with the best diagnostic performance compared to histopathology
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Diagnostic performance of the first postoperative value of urine IL-18, urine NGAL, and plasma NGAL for the detection of AKI.

Sensitivity

1-Specificity

Urine IL-18 (pg/mL) 0.74
Urine NGAL (ng/mL) 0.67
Plasma NGAL (ng/mL) 0.70
Potential Context of Interpretation of AKI Markers

- **Tubular Damage**
  - KIM-1, L-FABP, α-GST (albumin and NGAL)

- **Inflammation**
  - NGAL, IL-18

<table>
<thead>
<tr>
<th>Injury Marker</th>
<th>Inflammation Marker</th>
<th>GFR Marker</th>
<th>? Kidney Injury</th>
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<tbody>
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<td>+</td>
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Perfect Biomarker Performance in Setting of Imperfect Gold Standard

A. Actual disease prevalence = 20%. Red squares represent true disease positives. Blue squares represent true disease negatives.

B. Imperfect gold standard sensitivity = 80%. Of the 20 true disease positives, 4 are classified as disease negative by the imperfect gold standard (blue hatched squares).

True Negatives
True Positives

Prevalence = 20 % (20/100)

Imperfect gold standard picks up 16 of 20 true positives

Imperfect gold standard picks up 64 of 80 true negatives

Imperfect Gold Standard Sensitivity=80%

Perfect Biomarker Sensitivity=50% (16 of 32)
Perfect Biomarker Specificity = 94% (64 of 68)
Effect of Prevalence of AKI on Apparent Specificity and Sensitivity of a Perfect Biomarker when AKI Definition is Assumed 95% Sensitive and 90% Specific
## Plasma NGAL as a Predictor of AKI and Clinical Outcomes

<table>
<thead>
<tr>
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<th>ROC analysis</th>
<th>At cutoff of 150 ng/ml</th>
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<tr>
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<td>AuC ROC (95% CI)</td>
<td>Sensitivity (95% CI)</td>
</tr>
<tr>
<td>AKI within 48 h</td>
<td>0.78 (0.65–0.90)</td>
<td>0.73 (0.45–0.92)</td>
</tr>
<tr>
<td>AKI within 5 days</td>
<td>0.67 (0.55–0.79)</td>
<td>0.46 (0.27–0.67)</td>
</tr>
<tr>
<td>RRT within ICU stay</td>
<td>0.82 (0.70–0.95)</td>
<td>0.87 (0.60–0.98)</td>
</tr>
<tr>
<td>ICU mortality</td>
<td>0.67 (0.58–0.77)</td>
<td>0.60 (0.45–0.73)</td>
</tr>
<tr>
<td>RRT + ICU mortality</td>
<td>0.68 (0.59–0.77)</td>
<td>0.60 (0.47–0.73)</td>
</tr>
</tbody>
</table>

301 ICU patients; 133 (44%) developed AKI during ICU stay

Cruz...Ronco  Intensive Care Med.36:444-451, 2010
ROC curve for peak post-operative SCr after cisplatin therapy for mesothelioma (using 24h KIM-1 ≥ 2 ng/mg Ucr as gold standard)

AUC-ROC = 0.55

Very good biomarker

Serum Creatinine

AUC-ROC = 0.55
Admission-to-discharge percentage change in GFR grouped by presence or absence of hemoconcentration.


Copyright © American Heart Association
Survival curves grouped by presence or absence of hemoconcentration after adjustment for baseline characteristics.

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Cisplatin Study

A Prospective Observational Study of Urinary Biomarkers of Nephrotoxicity in Cancer Patients Receiving Cisplatin Chemotherapy

They will assess the performance of a large panel of kidney biomarkers in patients with various cancers who are scheduled to receive high-dose cisplatin chemotherapy and compare these with cancer control patients. Results from this study will be used to support the clinical qualification of biomarkers for drug-induced kidney injury.
Scheduled Visits & Sample Collection Time-Points

Pre-Tx

Day Screening

Day 0

Day 1 (<12 hours)

Day 4 + 1

Day 7 + 1

Cis.

Day 14 + 2

Day 21 + 4

Follow-up phase

Urine & Blood samples

(serum BUN, creatinine, cystatin C, sodium, and spot urine samples for novel biomarkers)
The Creatinine Normalization Issue

Waikar et al. Kidney Int. 2010
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- Produced by a specific segment of the kidney tubule or kidney endothelium
- Stable in the blood or urine, devoid of interferences with other substances, and unaffected by chemical composition of the urine, including ionic strength and pH among others
- Levels are predictive of clinically significant outcome
- Prognostic: Levels predict rapid progressors from others so that aggressive therapies can be personalized and clinical trials of new therapeutics can be facilitated.
- Progression: Levels reflect change in disease state over time. Usually require repeated measurements.
- Understandable function of the marker in the kidney
Conceptual Framework for the CKD Biomarker (CKDBiocon) Consortium
AKI Leads to CKD

CKD Predisposes to AKI
Many Factors contribute to the Progression of Kidney Disease

Tubular Injury, Oxidative Stress, Endothelial Injury, Fibrosis, Metabolic Dysfunction, Systemic Inflammation, Glomerular Injury

The more biomarkers we have for these pathways the more likely we are to be able to predict rates of progression and intervene effectively to alter the outcome.
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Biomarkers Reveal Important Aspects of Underlying Pathophysiological Disease Processes
Area under Receiver Operator Curve for Several Biomarkers for Development of KDIGO stage 2 or 3 within 12 hours of Sample Collection

Kashani et al Critical Care 17:R25, 2013
Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury

Kashani et al. Critical Care 17:R25, 2013
In Vivo Cell Cycle Analysis

Preparation for mitosis 19%
DNA synthesis 39%
Mitosis 3%
Quiescence (variable)
Synthesis required for DNA synthesis 40%

Ki67
G2/M
S
G1

BrdU uptake

Yang et al. Nature Medicine 2010
In Vivo Cell Cycle Analysis in AKI

Moderate IRI

Unilateral IRI

AAN

Yang et al. Nature Medicine 2010
Plasma KIM-1 is elevated in I/R mice

Plasma Creatinine

Plasma KIM-1

Absolute Urinary KIM-1

Normalized Urinary KIM-1

* P<0.001
Elevation of Plasma KIM-1 in patients who developed AKI after CPB

Normalized urinary KIM-1

Plasma KIM-1

# p<0.05, significant difference from baseline
* p<0.05, significant difference between AKI and non-AKI group (N=9)
How can Novel Biomarkers, assuming they bring new information about Kidney Disease, be a “False Dawn?”

• Their insight into pathophysiology is unappreciated and unincorporated into our approaches to therapies. How can we ignore new, easy to obtain information about pathophysiology, when we have no therapies???

• We do not spend enough effort understanding the origins, specificity and insight into kidney pathobiology they reveal.

• We rely on inadequate “gold standards” to validate the biomarker.

• In the case of CKD we are likely asking too much of a single biomarker given the complexity of disease process and the multiple factors that affect progression over time.
Biomarkers Provide Much More Insight Than This
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