Improving the prediction of disease progression: The search for improved markers of CKD

30th Arnold O. Beckman Conference
2013 Kidney Week, Atlanta, GA
November 5-6, 2013

Chi-yuan Hsu, MD, MSc
Professor and Chief
UCSF Division of Nephrology
Disclosures

• Urine biomarker assays (NGAL) donated Abbott (in CKD Biomarker Consortium)
Topics

• Examining the role of “AKI biomarkers” in CKD progression
• Lessons learnt
• Future opportunities
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Recent data: AKI accelerates progression/development of CKD

Amdur KI 2009
AKI-CKD connection

• “the AKI-CKD connection is of far reaching clinical significance....Identification of the AKI-CKD nexus represents the single most important advance in understanding of the mechanisms of progression since hyperfiltration was shown to occur following renal ablation.”

Venkatachalam Am J Physiol Renal Physiol 2010
Old paradigm: The trajectory of CKD is relatively smooth.
New paradigm: The course of CKD is punctuated by episodes of AKI

Graph 1: Loss of eGFR after AKI event, but eGFR slope stays the same.

Graph 2: eGFR slope decreases after AKI event.
Motivation for this line of investigation

- If clinically evident cases of AKI accelerate the progression/development of CKD, are more subtle episodes of injury—detected by “AKI biomarkers”—also important?
- Candidate injury biomarkers include neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1)
Neutrophil gelatinase-associated lipocalin (NGAL)
"AKI biomarkers" are detectable in CKD

- Urine NGAL measured in baseline urine samples among 3386 Chronic Renal Insufficiency Cohort (CRIC) study participants (mean eGFR 42 ml/min/1.73m$^2$; 1.1 gm proteinuria)
- Median concentration 17.2 ng/mL (IQR 8.1-39.2 ng/mL)(5% between 178.9-3069.6 ng/mL)
- Higher NGAL concentration among those who are female, non-white, with lower eGFR, higher proteinuria, DM, higher BP
Higher urine NGAL an independent risk factor for ESRD/eGFR halving

<table>
<thead>
<tr>
<th>Quintiles of baseline urine NGAL concentration (ng/ml)</th>
<th>Events</th>
<th>Rate (per 100 person-years)</th>
<th>Unadjusted HR (95% confidence intervals)</th>
<th>Additionally adjusted for other baseline covariates&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤6.9</td>
<td>47</td>
<td>1.9</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>&gt;6.9 to ≤12.9</td>
<td>77</td>
<td>3.3</td>
<td>1.75 (1.22-2.51)</td>
<td>1.37 (0.94-1.98)</td>
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<tr>
<td>&gt;12.9 to ≤22.6</td>
<td>105</td>
<td>4.8</td>
<td>2.52 (1.79-3.56)</td>
<td>1.24 (0.86-1.79)</td>
</tr>
<tr>
<td>&gt;22.6 to ≤49.5</td>
<td>173</td>
<td>8.1</td>
<td>4.30 (3.11-5.93)</td>
<td>1.39 (0.97-2.00)</td>
</tr>
<tr>
<td>&gt;49.5</td>
<td>287</td>
<td>16.9</td>
<td>9.34 (6.86-12.72)</td>
<td>1.70 (1.16-2.48)</td>
</tr>
</tbody>
</table>

HR per 1 unit increase in log (urine NGAL) 1.75 (1.66-1.85) 1.11 (1.01-1.21)  
P<0.0001  
P = 0.0230

<sup>a</sup> age, sex, race/ethnicity, eGFR, 24-hr urine protein, DM, SBP, BMI, use of ACE-I/ARB, education attainment, hx of CVD

Liu KI 2013
Effect strongest in the first 2 years of follow-up

Category-free NRI 24.7% (0.4 to 38.5%)

Figure 2 | Multivariable-adjusted hazard ratio (per increase in log urine neutrophil gelatinase-associated lipocalin concentration) overall and by duration of follow-up.
[FR-PO275] Urinary Biomarkers as Risk Factors of End-Stage Renal Disease in the General Population: CKD Biomarkers Consortium

Meredith C. Foster, Josef Coresh, MD, PhD, FASN, Joseph V. Bonventre, MD, PhD, FASN, Chi-yuan Hsu, MD, Paul L. Kimmel, MD, FASN, Theodore E. Mifflin, PhD, Robert G. Nelson, MD, PhD, Vasan S. Ramachandran, Venkata Sabbisetti, PhD, Sushrut S. Waikar, MD, Kathleen D. Liu, MD.
Median time between biomarker measurement and ESRD event = 6 years
[FR-PO278] Association of Urinary LFABP, KIM-1, and NAG with Incident End-Stage Renal Disease and Mortality in Type 2 Diabetes

Gudeta D. Fufaa, PhD, E. Jennifer Weil, MD, Robert G. Nelson, MD, PhD, Joseph V. Bonventre, MD, PhD, FASN, Venkata Sabbisetti, PhD, Sushrut S. Waikar, MD, Theodore E. Mifflin, PhD, Xiaoming Zhang, Dawei Xie, Harold I. Feldman, MD, FASN, Josef Coresh, MD, PhD, FASN, Vasan S. Ramachandran, Paul L. Kimmel, MD, FASN, Chi-yuan Hsu, MD, Kathleen D. Liu, MD.
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
<th>Per SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIM-1/Cr</td>
<td>Reference</td>
<td>0.87 (0.44-1.72)</td>
<td>0.96 (0.48-1.94)</td>
<td>1.06 (0.77-1.45)</td>
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<tr>
<td>NAG/Cr</td>
<td>Reference</td>
<td>0.69 (0.28-1.70)</td>
<td>1.83 (0.75-4.44)</td>
<td>1.72 (1.11-2.68)</td>
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<tr>
<td>NGAL/Cr</td>
<td>Reference</td>
<td>3.62 (1.48-8.85)</td>
<td>7.10 (2.88-17.51)</td>
<td>1.85 (1.38-2.50)</td>
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<tr>
<td>LFABP/Cr</td>
<td>Detected vs. undetectable</td>
<td>0.79 (0.39-1.57)</td>
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ESRD Hazard ratios adjusted for DM duration, demographics, HTN, HbA1c, GFR, ACR

Median time between biomarker measurement and ESRD event = 9 years
Summary

• Consist with our hypothesis, some “AKI biomarkers” are independent risk factors of more rapid progression of CKD

• Little improvement in prediction of outcome events/reclassification

• Insight: effect is most notable with a 1-2 yr time horizon?

• This may make sense patho-physiologically and would need to be considered in future studies
• Examining the role of “AKI biomarkers” in CKD progression

• **Lessons learnt**

• Future opportunities
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1. Importance of urine handling

[FR-PO023] Urine Stability Studies for Novel Biomarkers of Acute Kidney Injury

Chirag R. Parikh, MD, PhD, FASN, Isabel Butrymowicz, MD, Angela Yu, Vernon M. Chinchilli, PhD, Meyeon Park, MD, Chi-yuan Hsu, MD, William Brian Reeves, MD, FASN, Prasad Devarajan, MD, Paul L. Kimmel, MD, FASN, Edward D. Siew, MD, Kathleen D. Liu, MD.

In press AJ KD; Haase-Fielitz AJ KD 2009
2. Importance of analytical chemistry lab QC

"The Westgard multirule system"
Lower limits of blank vs. detection vs. quantification
Proficiency samples (high/med/low)
3. Importance of managing information flow
“Informatics” problems

• Misunderstanding: data transmitted incorrectly in part because the working sample manifest sent to performance lab was INVERTED relative to the sample packing order
Template A. Samples were placed in this format (Standard Procedure Template):

- **S** Standards
- **B** Blanks
- **C** Controls
- **X** Unknown Samples
- **17 18 19** Proficiency Samples
Template B. Data was reported as if the samples were arranged in this order:

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<td>8</td>
<td>8</td>
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</tbody>
</table>
4. Importance of constant vigilance

Pearson r = 0.6357

Courtesy: Robert Nelson, MD, NIDDK
water loss = old UCr/new UCr

Courtesy: Robert Nelson, MD, NIDDK
Corrected for Water Loss

Pearson $r = 0.9362$

Courtesy: Robert Nelson, MD, NIDDK
Cryovial without neoprene/silicon gasket

Cryovial with gasket

Not a cryovial

Courtesy: Robert Nelson, MD, NIDDK
Diagnosis: desiccation

• Problem with urine stored for ~20 years at -80°C when not using cryovials with gaskets
• Outcome: express urine biomarkers as per gm urine creatinine
• Measure urine biomarkers after dilution (1:4)(“sticky” samples with bead aggregation on Luminex platform)
5. Importance of blind replicates
Blind replicates biomarker/Cr ratios were better correlated
Diagnosis: incomplete mixing

- After more investigation, including measuring other daughter tubes, we concluded that the problem was due to incomplete mixing of the large 9 cc mother tube

- Outcome: about one-quarter of the samples require re- aliquoting in order to have daughter tubes suitable for biomarker studies
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Much remains to be learned

<table>
<thead>
<tr>
<th></th>
<th>ARIC controls (N=192)</th>
<th>Pima normo-albminuria group (N=138)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>51%</td>
<td>100%</td>
</tr>
<tr>
<td>GFR</td>
<td>70 ml/min/1.73m² (CKD-EPI)</td>
<td>153 ml/min (measured)</td>
</tr>
<tr>
<td>Urinary Alb/Cr</td>
<td>13 [3-108]</td>
<td>8 [5-14]</td>
</tr>
<tr>
<td></td>
<td>median [IQR] (mg/gm)</td>
<td></td>
</tr>
<tr>
<td>KIM-1/Cr</td>
<td>659 [361-1047]</td>
<td>449 [169-997]</td>
</tr>
<tr>
<td></td>
<td>median [IQR] (ng/gm)</td>
<td></td>
</tr>
<tr>
<td>NGAL/Cr</td>
<td>15 [9-40]</td>
<td>44 [18-80]</td>
</tr>
<tr>
<td></td>
<td>median [IQR] (μg/gm)</td>
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</tr>
</tbody>
</table>
48,242 aliquots made (34,954 in CKD BioCon central lab)
Within a prospective cohort study, a nested case-cohort design is an efficient way to validate novel biomarkers longitudinally.
Summary

• Some signal that “AKI biomarkers” are important with regard to the progression of CKD (especially within a 1-2 yr time horizon)

• There are many factors that need to be considered in order to optimally test the performance of novel biomarkers in large scale human studies

• Much remains to be learned but future studies may be made easier as a result of the infrastructure built by groups such as the CKD Biomarker Consortium
Acknowledgement

• NIH-NIDDK
• Kathleen Liu
• Robert Nelson, Joseph Bonventre, Venkata Sabbisetti, Theodore Mifflin, Dawei Xie, Sus Waikar, Joe Coresh, Meredith Foster, Alan Go, Harold Feldman, Chirag Parikh, Vasan Ramanchandran