MARKERS OF ACUTE KIDNEY INJURY
Applying theory to practice

Soto K, Frade F, Papoila AL, Tiago Ribeiro et al.

Servico de Nefrologia e de Urgencias
HOSPITAL FERNANDO FONSECA
DEPARTAMENTO DE BIOESTATISTICA DA UNIVERSIDADE NOVA, DE LISBOA
INSTITUTO SUPERIOR DE ENGENHARIA DE LISBOA
CARES

REVIEWING OUR KNOWLEDGE

CHANGING CONCEPTS

UNDERSTANDING ABOUT BIOMARKERS

MOVING FORWARD

FROM BEDSIDE TO BENCH...
## Demonstrative ED cases

<table>
<thead>
<tr>
<th>AGE</th>
<th>COMORB</th>
<th>CKD</th>
<th>CVD</th>
<th>DM</th>
<th>CAUSE of ADM</th>
<th>no-RENAL DIAGNOSTIC</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>HIPOGLICEMIA</td>
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<td>2</td>
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<td>4</td>
<td>1</td>
<td>1</td>
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<td>ANGOR INST</td>
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<td>0</td>
<td>0</td>
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<td>4</td>
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<td>1</td>
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<td>0</td>
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<td>79</td>
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<td>0</td>
<td>DIARREIA</td>
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<td>0</td>
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<td>HIPOGLICEMIA</td>
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</tbody>
</table>

Demonstrative ED cases!
# Cases: past history and outcome

<table>
<thead>
<tr>
<th>Baseline</th>
<th>admission</th>
<th>SUSCEPTIBILITY</th>
<th>SCR_S1</th>
<th>CYC_S1</th>
<th>AKI/TAZ</th>
<th>DIALYSIS</th>
<th>ICU</th>
<th>ALL SEPSIS</th>
<th>DEAD1</th>
<th>DIAG</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>B_SCR</td>
<td>B_eGFR</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>1,5</td>
<td>49,85</td>
<td>4</td>
<td>2,5</td>
<td>2,65</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>2</td>
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<td>1,8</td>
<td>35,53</td>
<td>4</td>
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<td>1,73</td>
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<td>0</td>
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<tr>
<td>3</td>
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<td>0,7</td>
<td>120,49</td>
<td>1</td>
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<td>1,28</td>
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<td>0</td>
<td>0</td>
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<td>4</td>
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<td>79,11</td>
<td>2</td>
<td>1,6</td>
<td>1,38</td>
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<td>1</td>
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<td>2,46</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

**Past history and outcome**

- **Cases**:
  - B_SCR: Baseline serum creatinine
  - B_eGFR: Baseline estimated glomerular filtration rate
  - SCR_S1: SCR at admission
  - CYC_S1: CyC at admission
  - AKI/TAZ: AKI/TAZ at admission
  - DIALYSIS: Dialysis treatment
  - ICU: Intensive care unit stay
  - ALL SEPSIS: All sepsis
  - DEAD1: Dead 1
  - DIAG: Diagnosis

**Diagnosis Options**:
- FMO: Focal Motor Cortex
- SEPSIS: Sepsis
## Cases: long term outcome

<table>
<thead>
<tr>
<th></th>
<th>SCr D</th>
<th>GFR_D</th>
<th>1FU Scr</th>
<th>1FU GFR</th>
<th>FU month</th>
<th>death FU</th>
<th>CKD + AKI</th>
<th>Last Scr</th>
<th>Last GFR</th>
<th>FU y</th>
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<tbody>
<tr>
<td>1</td>
<td>2,7</td>
<td>25,3</td>
<td>3,2</td>
<td>20,79</td>
<td>12,7</td>
<td>0</td>
<td>CKD 5</td>
<td>5,50</td>
<td>11,00</td>
<td>3,0</td>
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<tr>
<td>2</td>
<td>3,1</td>
<td>19,0</td>
<td>5</td>
<td>10,93</td>
<td>26,0</td>
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<td>3</td>
<td>1,6</td>
<td>46,4</td>
<td>6</td>
<td>10,10</td>
<td>2,4</td>
<td>1</td>
<td>CKD+AKI HD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7,4</td>
<td>7,9</td>
<td></td>
<td>0,3</td>
<td></td>
<td></td>
<td>AKI</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5</td>
<td>2,1</td>
<td>38,9</td>
<td>7,9</td>
<td>8,44</td>
<td>10,3</td>
<td>0</td>
<td>CKD5 HD</td>
<td></td>
<td></td>
<td>3,0</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>76,7</td>
<td>10,3</td>
<td>5,20</td>
<td>1,0</td>
<td>1</td>
<td>CKD+AKI HD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2,9</td>
<td>22,9</td>
<td>5,6</td>
<td>10,71</td>
<td>1,0</td>
<td></td>
<td>CKD+AKI HD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Grades of susceptibility

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>eGFR &gt; 90ml/ min/ 1.73 m²</td>
</tr>
<tr>
<td>2</td>
<td>Previous CKD 2</td>
</tr>
<tr>
<td>3</td>
<td>Previous CKD 3</td>
</tr>
<tr>
<td>4</td>
<td>Previous CKD 2/3 +</td>
</tr>
<tr>
<td></td>
<td>- DM with microalbuminuria</td>
</tr>
<tr>
<td></td>
<td>- Dehydration</td>
</tr>
<tr>
<td></td>
<td>- MM</td>
</tr>
<tr>
<td></td>
<td>- HF</td>
</tr>
<tr>
<td></td>
<td>- Decompensate cirrhosis</td>
</tr>
</tbody>
</table>

Biomarkers for AKI diagnosis
Reviewing

Limitations of SCr

Serum creatinine fall

production

lean body mass
dietary intake
liver disease
Ther Drug Monit 5: 1983

extensive loss of hepatocyte glycogen stores
Kidney Int 69: 2006

Renal clearance
Extra-renal clearance
Degradation
Gastrointestinal excretion
Clin Sci (Lond) 58:1980

loss of muscle mass + reduce muscle production
(hormonal and inflammatory mediators)
Lancet 2004

Sepsis-induced hypothermia decrease nonenzymatic conversion of creatine to creatinine
Infect Immun 67: 6603–6610, 1999
Changing concepts

RIFLE criteria for diagnosing AKI

Changing concepts

**AKIN staging system for AKI**

<table>
<thead>
<tr>
<th>AKIN stage</th>
<th>Serum Creatinine Criteria</th>
<th>Urinary Output Criteria</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>▲ Cr ≥ 0.3 mg/dL or ▲ ≥ 150-200% from baseline</td>
<td>&lt; 0.5 mL/kg/hr</td>
<td>&gt; 6 hrs</td>
</tr>
<tr>
<td>2</td>
<td>▲ Cr to &gt; 200-300% from baseline</td>
<td>&lt; 0.5 mL/kg/hr</td>
<td>&gt; 12 hrs</td>
</tr>
<tr>
<td>3</td>
<td>▲ Cr to &gt; 300% from baseline or Cr ≥ 4mg/dL with an acute rise of at least 0.5 mg/dL</td>
<td>&lt; 0.5 mL/kg/hr or anuria</td>
<td>X 24 hrs X 12 hrs</td>
</tr>
</tbody>
</table>

*Patients needing RRT are classified stage 3 despite the stage they were before starting RRT*

## Cases: AKI diagnosis

<table>
<thead>
<tr>
<th>s</th>
<th>0 H</th>
<th>6H</th>
<th>12H</th>
<th>24H</th>
<th>48H</th>
<th>1W</th>
<th>DISCH</th>
<th>AKIN</th>
<th>death FU</th>
<th>CKD + AKI</th>
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</thead>
<tbody>
<tr>
<td>4</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>1</td>
<td>0</td>
<td>CKD 5</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>1</td>
<td>2Y</td>
<td>CKD5 HD</td>
</tr>
<tr>
<td>1</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>R</td>
<td>I</td>
<td>O</td>
<td>I</td>
<td>2</td>
<td>1Y</td>
<td>CKD+AKI HD</td>
</tr>
<tr>
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<td>R</td>
<td>0</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>F</td>
<td>F</td>
<td>3</td>
<td>INT</td>
<td>AKI</td>
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<tr>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>O</td>
<td>1</td>
<td>0</td>
<td>CKD5 HD</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>R</td>
<td>R</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1Y</td>
<td>CKD+AKI HD</td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>INT</td>
<td>CKD+AKI HD</td>
</tr>
</tbody>
</table>
About Biomarkers

Conceptual model of AKI

About Biomarkers

- Early prediction and diagnosis of AKI (before SCr)
- Identify the primary location of injury
- Discriminate PreRenal, CKD and AKI
- Prediction of severity
- Identify the etiology: ischemic, toxic, septic, combination


Mehta RL et al. (2007) Crit Care 11: R31

Koyney and Murray, Argutus Medical
About Biomarkers for AKI diagnosis

Sources: Coca et al,2 Parikh et al,3 Bagshaw et al.4 Belcher et al, AJKD 2011
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Origin in AKI cases</th>
<th>Significance of rise</th>
<th>Studied clinical settings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NGAL</strong> serum and urinary</td>
<td>Urine: synthesis in <em>distal nephron</em> and secreted into urine Circulating: synthesized <em>systemically</em>, filtered, and uptaken by proximal tubular cells with a little amount secreted in the urine</td>
<td>Tubular injury (ischemia and nephrotoxins)</td>
<td>(i) Early detection of AKI after cardiac surgery, ICU, ED, and after nephrotoxins (ii) Risk stratification (iii) Prognostic marker after kidney transplantation (iv) Monitoring interventional trials in AKI (v) Prognosis of RRT and mortality</td>
</tr>
<tr>
<td><strong>CysC</strong> serum and urinary</td>
<td>Produced at a constant rate by <em>nucleated cells</em>, filtered, and almost completely reabsorbed in the proximal tubules</td>
<td>Change in GFR (proximal tubule injury)</td>
<td>(i) Early detection of AKI after cardiac surgery, ICU, ED and after nephrotoxins (ii) Prognosis of RRT and mortality</td>
</tr>
<tr>
<td><strong>KIM-1</strong> urinary</td>
<td>Type 1 transmembrane protein, highly expressed in dedifferentiated <em>proximal tubule</em> epithelial cells after ischemic or toxic injury and is not detectable in normal tissue</td>
<td>Tubular injury (ischemia and nephrotoxins)</td>
<td>(i) Early detection of AKI after cardiac surgery and after nephrotoxins (ii) Prognosis of RRT and mortality</td>
</tr>
<tr>
<td><strong>IL-18</strong> urinary</td>
<td>Proinflammatory cytokine originates from <em>tubular epithelial</em> cells</td>
<td>Tubular injury (ischemia and nephrotoxins)</td>
<td>(i) Early detection of AKI after cardiac surgery, in ICU (ii) Prognostic marker after kidney transplantation (iii) Prognosis of mortality</td>
</tr>
</tbody>
</table>
CYSTATIN C

- Cystatin C production is independent from muscle mass, constitution of the body, or nutrition
- It is independent or from gender and age
- Probably it is influenced by inflammation and malignancy
- It is influenced by very large doses of glucocorticoids and thyroid dysfunction

- free renal filtration
- no tubular secretion
- no re-entry into circulation
- tubular reabsorption followed by degradation

http://structbio.nature.com
Cystatin C for differential diagnosis

![Graph showing SCyst C (mg/l) vs. Classification (1-AKI, 2-preR, 3-CKD, 4-NF)]

- AKI
- Pre-R
- CKD
- NF

\[ p < 0.001 \]

![Graph showing SCysC (g/L) vs. Time of study (hours)]

- AKI
- Non-AKI

\[ p < 0.001 \]

Soto K et al. CJASN 2010
Cystatin C as marker of severity

RIFLE and GFR based on SCysC

AKIN and levels of SCysC

Soto K et al. CJASN 2010
NGAL

Neutrophil gelatinase-associated lipocalin

- First identified as a neutrophil granule protein
- Expressed at very low levels on several tissues
- Normally very small amounts in kidney tubules
- Induced in injured epithelial cells
- The most upregulated gene in the kidney, very early after ischemic or nephrotoxic AKI in animals

NGAL for AKI diagnosis

Soto K et al. 2009

NGAL for AKI diagnosis!

![Graph showing NGAL levels in different classifications.](image)

**Classification (1-AKI, 2-preR, 3-CKD, 4-NF)**

- AKI
- Pre-R
- CKD
- NF

**SNGAL (ng/mL)**

- AKI
- Pre-R
- CKD
- NF

**NGAL/Cr (ng/mg creat)**

- AKI
- Pre-R
- CKD
- NF

**UNGAL/Creat (ng/mg creat)**

- AKI
- Pre-R
- CKD
- NF

**p <0,001**
NGAL and accurance

Comparison with Scr

Marker of AKI on CKD
Urine sediment score to quantitatively evaluate AKI

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RTE cells 0 and granular casts 0</td>
</tr>
<tr>
<td>2</td>
<td>RTE cells 0 and granular casts 1 to 5 or RTE cells 1 to 5 and granular casts 0</td>
</tr>
<tr>
<td>3</td>
<td>RTE cells 1 to 5 and granular casts 1 to 5 or RTE cells 0 and granular casts 6 to 10 or RTE cells 6 to 20 and granular casts 0</td>
</tr>
</tbody>
</table>

*Urine profile in ATN:*

* renal tubular epithelial cells

* coarse granular, muddy brown

or mixed cellular casts

*Changing concepts*

Perazella MA, G. Coca S CJASN 2012;7:167-174
Biomarkers not only for accurate diagnosis

Primary outcome occurrence: worsened AKI or in-hospital death

Urine biomarker groups (NGAL, IL-18, KIM-1 in quartiles and urine microscopy score of 0, 1, 2, ≥3)

Perazella MA, G. Coca S CJASN 2012;7:167-174
BIOMARKERS EVALUATION

Ana Luisa Papoila, Faculdade de Ciências Médicas da UNL, CEAUL, Portugal
“biological characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes or pharmacologic response to therapeutic interventions”.

Understanding

Biomarkers

Classification and prediction
Classification and prediction

- **Diagnostic markers**
  Used in people with signs or symptoms, to aid in assessing whether they have the condition under study

- **Screening markers**
  Used in asymptomatic people, to detect a disease or condition at an early stage

- **Prognostic markers**
  Used in subjects diagnosed with a condition, to predict subsequent outcomes, such as disease relapse or progression
Model ‘the risk of disease” (disease outcome) with, for instance, logistic (or Cox) regression. A marker is considered useful if it has a strong effect on risk.

Evaluate classification performance – Receiver Operating Characteristic Curve (ROC)

DisCRETIZATION PROCEDURE

Sensitivity, specificity, predictive values and likelihood ratios
Results from these two evaluations may be apparently contradictory: A marker that is a strong predictor of risk may have a poor discriminative performance.

Ying Huang et al. 2007. Evaluating the predictiveness of a continuous marker. *Biometrics* 63,
Understanding

Risk prediction

- **S Creatinine**
  - AUC = 0.88
  - 95% CI: 0.85-0.92

- **S Cystatin C**
  - AUC = 0.87
  - 95% CI: 0.83-0.90

- **U CysC**
  - AUC = 0.61
  - 95% CI: 0.55-0.67
Understanding

SCysC: Cupoint = 0.98
Sensitivity: 81.40%
Specificity: 76.70%
PPV: 48.20%
NPV: 93.90%
LR (+): 3.49
LR (-): 0.24
Understanding

Application to risk prediction for AKI markers

markers: Cystatin_S1, Cystatin_U1

Prevalence of AKI = 21%
Changing concepts

Natural History of AKI leading to chronic disease

Partial recovery and No-recovery is more frequent than we thought.

AKI

Discharge

D Az

2Y follow-up
Changing concepts

Renal survival decrease after an AKI episode

Survival to CKD3

Survival to CKD4

Soto et al. 2012
Searching for predictors of renal outcome

pNGAL and CysC as a biomarkers of kidney evolution

Soto et. al 2012
And now?
NEFROLAB

OK
LET'S MOVE FORWARD
for homework:

- change our point of view about AKI, as a continuous disease that leads to renal imprint
- we have a lot to do, let’s start using clinical markers
- Applying concepts to clinical practice
- following those patients emphasizing nephroprotection
- let’s work together searching for solutions

thanks for sharing our concerns