Interest of NGAL as early marker of Acute Kidney Injury

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Content

• What is NGAL?
• What is Acute Kidney Injury (AKI)? Causes?
• What are the current tools for AKI assessment?
• Clinical interest of NGAL
• What are the analytical methods available (plasma, urine)?
• Interest in predicting Acute Tubular Necrosis after kidney transplantation
What is NGAL?
Neutrophil Gelatinase-Associated Lipocalin

- NGAL, a 25 kDa protein, belongs to the lipocalin superfamily, carrying ligands or siderophores (iron binding chemicals)
- Known to play a protective role against bacteria infections preventing microbial access to iron
- Originally characterized in neutrophils, but produced and expressed at very low levels normally by other tissues (kidney, prostate, arterial endothelium, atheromatous plaques, lungs, stomach, and colon...)

Devarajan, Expert Opin Med Diag 2008. 2 (4); 387-398
What is NGAL?

Neutrophil Gelatinase-Associated Lipocalin

• “Protective” role against renal injury and ischemia
  • Increased after injury
  • Increased after ischemia
  • One of the most highly induced proteins in kidney after ischemic or nephrotoxic AKI in animal models

• One of the earliest gene activated in case of nephrotoxic and ischaemic injuries
  • NGAL is rapidly up-regulated (few hours)
  • Induction of NGAL mRNA and protein in the kidney is a log order of magnitude, reaching a 1000 fold in the most severe cases

• Early diagnostic biomarker for AKI
Induction of Mouse Kidney NGAL After Ischemia

NGAL expression was highly induced after ischemia

Bilateral Ischemia Reperfusion

Con 3 12 24 48 72 Hours

Con = control

Ischemic Kidneys Synthesize Renal NGAL mRNA

mRNA Levels, fold change

Hours Post-Ischemia

*P ≤ 0.05

1000
100
10
1
0.1
0.01

NGAL
FGF2 (basic fibroblast growth factor)
HF (hypoxia-inducible factor)
BMP7 (bone morphogenic protein 7)

What is Acute Kidney Injury (AKI)?

- Formerly known as acute renal failure
- Abrupt decrease in kidney function that leads to accumulation of nitrogenous wastes, such as blood urea nitrogen and creatinine
- Revised nomenclature of AKI describes conditions that include both structural damage and dysfunction
- AKI is common
  - Definition of AKI is subjective and leads to difficulties in detection and diagnosis

What is Acute Kidney Injury (AKI)?

Incidence and prevalence

- Incidence ranges from 1% to 25% in developed countries
  - Incidence of renal replacement therapy (RRT) requirement ranges from 3.4% to 4.9%
  - Hospital mortality due to severe acute renal failure requiring RRT ranges from 60% to 70% in various countries
- Period prevalence in ICU
  - 5% to 6% requiring RRT in ICU
  - Overall hospital mortality was 60.3%

Common causes of AKI (hospital-acquired)

- Surgical procedures (e.g., CPB)
- Nephrotoxic agents (e.g., contrast)
- After renal Transplantation
- Sepsis, multi-system organ failure
- Critical illness (e.g., heart failure)

Adapted from Parikh and Devarajan, Crit Care Med 2008, 36:159-65
Contributing Factors to Acute Renal Failure

- Septic Shock: 48%
- Major Surgery: 34%
- Cardiogenic Shock: 27%
- Hypovolemia: 26%
- Drug-induced: 19%
- Hepatorenal Syndrome: 6%
- Obstructive Uropathy: 3%
- Other: 12%

RIFLE Criteria for Defining Acute Renal Failure

<table>
<thead>
<tr>
<th>Risk</th>
<th>GFR Criteria</th>
<th>Urine Output Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increased SCr x 1.5 or GFR decrease &gt;25%</td>
<td>&lt;0.5 mL/kg/h x 6 h</td>
</tr>
<tr>
<td>Injury</td>
<td>Increased SCr x 2 or GFR decrease &gt;50%</td>
<td>&lt;0.5 mL/kg/h x 12 h</td>
</tr>
<tr>
<td>Failure</td>
<td>Increased SCr x 3 or GFR decrease 75% or SCr ≥4 mg/dL*</td>
<td>&lt;0.3 mL/kg/h x 24 h or anuria x 12 hours</td>
</tr>
<tr>
<td>Loss</td>
<td>Persistent ARF = complete loss of kidney function &gt;4 weeks</td>
<td></td>
</tr>
<tr>
<td>ESKD</td>
<td>End stage kidney disease (&gt;3 months)</td>
<td></td>
</tr>
</tbody>
</table>

GFR = glomerular filtration rate, ARF = acute renal failure, SCr = serum creatinine
*With an acute rise >0.5 mg/dL

What are the current tools for AKI assessment?

- Creatinine (serum, urine)
- Urine output
- Cystatin C (serum/plasma)
- IL-18 (urine)
- KIM-1 (urine)
- NGAL (serum or urine)
What are the current tools for AKI?

Creatinine

- Typically used for diagnosis of acute injury ("standard")
- Marker of GFR not kidney injury
- Unreliable indicator during acute changes
  - Slow rate of changes
  - Concentrations may not change until about 50% of kidney function has already been lost
  - Does not accurately reflect kidney function until a steady state has been reached (may take up to 48 hours)
- Performs poorly during postoperative period

Evolution in Renal Diagnostics

<table>
<thead>
<tr>
<th>Time</th>
<th>AMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1950 ’s</td>
<td>WBC count</td>
</tr>
<tr>
<td>1960 ’s</td>
<td>LDH</td>
</tr>
<tr>
<td>1970 ’s</td>
<td>CPK</td>
</tr>
<tr>
<td>1980 ’s</td>
<td>CK-MB</td>
</tr>
<tr>
<td>1990 ’s</td>
<td>Troponin - T</td>
</tr>
<tr>
<td>2000</td>
<td>Troponin - I</td>
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</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>AKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1950 ’s</td>
<td>Change in serum creatinine</td>
</tr>
<tr>
<td>1960 ’s</td>
<td>No Change</td>
</tr>
<tr>
<td>1970 ’s</td>
<td></td>
</tr>
<tr>
<td>1980 ’s</td>
<td></td>
</tr>
<tr>
<td>1990 ’s</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>Change in serum creatinine</td>
</tr>
</tbody>
</table>

The renal testing arena is in need of the introduction of novel, early and more sensitive and specific biomarkers.

What are the current tools for AKI? Creatinine is not an ideal biomarker

- GFR (mL/min)
- Serum Creatinine (SCr) (mg%)
What are the current tools for AKI?

Cystatine C

- Cysteine protease inhibitor
- Synthesized and released into blood at relatively constant rate by all nucleated cells
- Measurement of GFR
  - Blood levels are not affected by age, gender, race, or muscle mass
  - Advantage in children, the elderly, intensive care unit patients, and high risk patients (diabetes)
- Few studies on use for AKI

What are the current tools for AKI?

Interleukin-18 (IL-18)

- Proinflammatory cytokine
- Induced and cleaved in the proximal tubule and detected in the urine following ischemic AKI
  - Detected 4–6 hours post-cardiopulmonary bypass
  - Detected in ICU setting 48 hours before AKI
- Early predictive marker for AKI within next 24 hours and predictor of mortality
  - Predicts development of AKI 24 hours before serum creatinine
- Can differentiate between acute tubular necrosis and other types of acute renal diseases
- May be influenced by other variables and in other pathophysiologic states

What are the current tools for AKI?

Kidney Injury Molecule 1 (KIM-1)

- Transmembrane protein, highly overexpressed in dedifferentiated proximal tubule cells after ischemic or nephrotoxic AKI
- Good at evaluating diagnosis of already established AKI
  - Not strong at facilitating early diagnosis of AKI
- Urine KIM-1 distinguished ischemic AKI from prerenal azotemia and chronic renal disease
- Can predict adverse clinical outcomes and is associated with measures of disease severity
  - Mortality risk prediction after AKI
- Very specific to ischemic or nephrotoxic injury, but not sensitive at earliest time points

What are the current tools for AKI?

NGAL

- More specific to AKI and can discern AKI subtypes
- Sensitive to establish an early diagnosis
  - Easily detected in urine and blood very soon after AKI
  - Earlier detection compared with other biomarkers
- Conserved across species
- Proportional increase with injury or loss of function
- Results predict clinical outcomes
  - Can be used as an early diagnostic factor and predictor of prognosis or severity of AKI

## New Biomarkers for AKI in Various Clinical Situations

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sample Source</th>
<th>Cardiopulmonary Bypass (CPB)</th>
<th>Contrast Nephropathy</th>
<th>Sepsis or ICU</th>
<th>Kidney Transplant (tx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C</td>
<td>Plasma</td>
<td>12 hrs post-CPB</td>
<td>8 hrs postcontrast</td>
<td>48 hrs before AKI</td>
<td>Variable</td>
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<tr>
<td>IL-18</td>
<td>Urine</td>
<td>4-6 hrs post-CPB</td>
<td>Not tested</td>
<td>48 hrs before 12-24 hrs post-tx AKI</td>
<td></td>
</tr>
<tr>
<td>KIM-1</td>
<td>Urine</td>
<td>12-24 hrs post-CPB</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Not tested</td>
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<tr>
<td>NGAL</td>
<td>Urine/Plasma</td>
<td>2 hrs post-CPB</td>
<td>2-4 hrs postcontrast</td>
<td>48 hrs before AKI</td>
<td>12-24 hrs post-tx (urine only)</td>
</tr>
</tbody>
</table>

Adapted from Parikh CR, Devarajan P. *Crit Care Med.* 2008;36(suppl):S159-S165.
Clinical Interest of NGAL

• Start citing references on PubMed from the early 90’
  – 25% of the reports were published in 2010!
• A major paper in Lancet in 2005 raised regain of interest
  – Mishra et al, Lancet, 2005, 365, 1231-8. NGAL as a biomarker for acute renal injury after cardiac surgery
• Important reports were published in different fields
  – Cardiac surgery and ICU (cardiopulmon bypass,…)
  – Septic patients
  – Kidney transplantation
  – Contrast media for coronary angiography, etc…
• A important review and Meta analysis has been recently published in Am J Kidney Dis in 2009
Clinical interest of NGAL
Mishra J et al Lancet 2005

Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery

- 71 children undergoing cardiopulmonary bypass surgery
- Serial urine and blood samples were analyzed for NGAL
- Primary outcome was acute renal failure defined as a 50% increase in serum creatinine from baseline
- 28% developed AKI
  - creatinine raised only 1-3 days after CPB
  - urine NGAL increased from 2 to 147 ng/mL after 2h (ROC 0.998, sensit 1.0, specif 0.98, cutoff 50 ng/mL
  - serum NGAL from 3 to 61 ng/mL after 2h
- NGAL powerful independent predictor of AKI
Clinical interest of NGAL
Mishra J et al Lancet 2005

Figure 1: Analysis of urine NGAL by western blot
(A) Representative western blot of urine samples obtained at various timepoints after cardiopulmonary bypass from a patient who subsequently developed acute renal failure. Both blots were probed with a monoclonal antibody to human NGAL. (B) Mean urine NGAL concentrations at various timepoints after cardiopulmonary bypass (upper) and creatinine (lower). Error bars are SE.

Figure 4: ROC curve analysis
Clinical interest of NGAL
Haase M et al meta-analysis Am J Kidney Dis 2009

ORIGINAL INVESTIGATIONS
Pathogenesis and Treatment of Kidney Disease

Accuracy of Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Diagnosis and Prognosis in Acute Kidney Injury: A Systematic Review and Meta-analysis

Michael Haase, MD,1 Rinaldo Bellomo, MD,2 Prasad Devarajan, MD,3 Peter Schliattmann, MD, MSc,4 and Anja Haase-Fiolitz, PharmD.1 and the NGAL Meta-analysis Investigator Group

American Journal of Kidney Diseases, Vol 54, No 6 (December), 2009: pp 1012-1024

• Previous conflicting results about the robustness of NGAL
  – Problem in definition of AKI among studies and timing for NGAL
• 19 studies involving 2538 patients of whom 487 → AKI
  – Urine and serum
  – Adults and pediatrics
  – Cardiac surgery
  – Critically ill patients
  – Contrast infusions
Clinical interest of NGAL
Haase M et al meta-analysis Am J Kidney Dis 2009

• The meta analysis used the RIFLE definition for AKI
• Recalculated data after contacting respective authors
• Time for determination of NGAL related to AKI event to evaluate performance
  – When the time of renal insult was known, refer to NGAL 6h after
  – When the time was unknown, refer to 24-48h before AKI diagnosis
• Conditions of storage was analysed
• Assessment of ROC curves, diagnostic odds ratio (DOR)
• Evaluation of the type of assays (manual vs automated)
• Performance according to age
Clinical interest of NGAL
Haase M et al meta-analysis Am J Kidney Dis 2009

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample Size</th>
<th>Population Type</th>
<th>Age (y)</th>
<th>Women (%)</th>
<th>Mean Baseline Serum Creatinine (mg/dL)</th>
<th>Impaired Renal Function (%)</th>
<th>Setting</th>
<th>NGAL Measurement</th>
<th>Country</th>
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<tbody>
<tr>
<td>Mishra et al, 2005</td>
<td>71</td>
<td>Children</td>
<td>3.0</td>
<td>36.6</td>
<td>0.45</td>
<td>0</td>
<td>CS</td>
<td>Plasma + urine</td>
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<td>Wagener et al, 2006</td>
<td>81</td>
<td>Adults</td>
<td>64.7</td>
<td>34.6</td>
<td>1.10</td>
<td>32.1</td>
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<td>Dent et al, 2007</td>
<td>123</td>
<td>Children</td>
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<td>0</td>
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<td>Children</td>
<td>7.1</td>
<td>48.7</td>
<td>0.44</td>
<td>0</td>
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<td>Hirsch et al, 2007</td>
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<td>6.9</td>
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<td>0.73</td>
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<td>CIN</td>
<td>Plasma + urine</td>
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<tr>
<td>Wagener et al, 2008</td>
<td>426</td>
<td>Adults</td>
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<td>33.8</td>
<td>1.08</td>
<td>27.2</td>
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<td>Urine</td>
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<td>Bennett et al, 2008</td>
<td>196</td>
<td>Children</td>
<td>4.0</td>
<td>46.4</td>
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<td>0</td>
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<td>Urine</td>
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<td>Ling et al, 2008</td>
<td>40</td>
<td>Adults</td>
<td>67.9</td>
<td>40.0</td>
<td>0.83</td>
<td>0</td>
<td>CIN</td>
<td>Urine</td>
<td>China</td>
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<td>Koyner et al, 2008</td>
<td>72</td>
<td>Adults</td>
<td>61.3</td>
<td>29.2</td>
<td>1.24</td>
<td>26.4</td>
<td>CS</td>
<td>Plasma + urine</td>
<td>United States</td>
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<td>Nickolas et al, 2008</td>
<td>541</td>
<td>Adults</td>
<td>59.2</td>
<td>48.4</td>
<td>1.20</td>
<td>26.8</td>
<td>ED</td>
<td>Urine</td>
<td>United States</td>
</tr>
<tr>
<td>Lima et al, 2008</td>
<td>52</td>
<td>Adults</td>
<td>54.7</td>
<td>42.3</td>
<td>1.20</td>
<td>53.8</td>
<td>CS</td>
<td>Urine</td>
<td>Brazil</td>
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<td>Wheeler et al, 2008</td>
<td>143</td>
<td>Children</td>
<td>2.2</td>
<td>28.0</td>
<td>0.76</td>
<td>—</td>
<td>ICU</td>
<td>Plasma</td>
<td>United States</td>
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<tr>
<td>Xin et al, 2008</td>
<td>33</td>
<td>Children + adults</td>
<td>38.0</td>
<td>42.4</td>
<td>0.77</td>
<td>0</td>
<td>CS</td>
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<td>China</td>
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<td>Cruz et al, 2009</td>
<td>301</td>
<td>Adults</td>
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<td>0.97</td>
<td>6.7</td>
<td>ICU</td>
<td>Plasma</td>
<td>Italy</td>
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<td>Makris et al, 2009 (CIN)</td>
<td>60</td>
<td>Adults</td>
<td>62.8</td>
<td>18.3</td>
<td>0.86</td>
<td>13.3</td>
<td>ICU</td>
<td>Plasma</td>
<td>Greece</td>
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<td>Makris et al, 2009 (ICU)</td>
<td>31</td>
<td>Adults</td>
<td>41.9</td>
<td>19.4</td>
<td>0.97</td>
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<td>ICU</td>
<td>Urine</td>
<td>Greece</td>
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<td>Tuladhar et al, 2009</td>
<td>50</td>
<td>Adults</td>
<td>66.7</td>
<td>30.0</td>
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<td>Plasma + urine</td>
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<td>Constantin et al, 2009</td>
<td>88</td>
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<td>57.0</td>
<td>45.5</td>
<td>0.81</td>
<td>—</td>
<td>ICU</td>
<td>Plasma</td>
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<td>Haase-Fiehlitz et al, 2009</td>
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<td>69.5</td>
<td>39.0</td>
<td>1.04</td>
<td>27.0</td>
<td>CS</td>
<td>Plasma</td>
<td>Australia</td>
</tr>
</tbody>
</table>

Note: Conversion factor for serum creatinine in mg/dL to μmol/L, ×88.4.

Abbreviations and definitions: CIN, contrast-induced nephropathy; CS, cardiac surgery-associated acute kidney injury; ED, emergency department; ICU, intensive care unit; NGAL, neutrophil gelatinase-associated lipocalin.
Clinical interest of NGAL
Haase M et al meta-analysis Am J Kidney Dis 2009

<table>
<thead>
<tr>
<th>Setting (no. of events/total patients; no. of studies [data sets])</th>
<th>Sensitivitya (95% CI)</th>
<th>Specificitya (95% CI)</th>
<th>DORa (95% CI)</th>
<th>AUC-ROCa (95% CI)</th>
<th>R² (%)</th>
<th>NGAL Cutoffa (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKI across settings (487/2,538; 19 [23])</td>
<td>76.4 (70.4-81.6)</td>
<td>85.1 (76.6-90.9)</td>
<td>18.6 (9.0-38.1)</td>
<td>0.815 (0.732-0.892)</td>
<td>43.5</td>
<td>190.2 (122.8-257.2)</td>
</tr>
<tr>
<td>AKI after cardiac surgery (307/1,204; 10 [13])</td>
<td>75.5 (70.2-82.4)</td>
<td>75.1 (65.2-86.3)</td>
<td>13.1 (5.7-34.8)</td>
<td>0.775 (0.669-0.867)</td>
<td>27.8</td>
<td>273.6 (145.0-289.2)</td>
</tr>
<tr>
<td>AKI in critically ill patients (123/602; 5 [5])</td>
<td>76.4 (59.9-87.5)</td>
<td>75.5 (52.2-89.7)</td>
<td>10.0 (3.0-33.1)</td>
<td>0.728 (0.615-0.834)</td>
<td>17.5</td>
<td>155.0 (150.8-169.0)</td>
</tr>
<tr>
<td>AKI after contrast infusion (34/191; 3 [4])</td>
<td>77.8 (62.8-88.0)</td>
<td>96.3 (74.4-99.6)</td>
<td>92.0 (10.7-794.1)</td>
<td>0.894 (0.826-0.950)</td>
<td>3.2</td>
<td>100.0 (80.0-100.0)</td>
</tr>
<tr>
<td>AKI in children across settings (213/663; 6 [8])</td>
<td>77.6 (69.7-83.9)</td>
<td>88.0 (75.8-94.5)</td>
<td>25.4 (8.9-72.2)</td>
<td>0.930 (0.883-0.968)</td>
<td>3.5</td>
<td>135.0 (50.0-150.0)</td>
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<tr>
<td>AKI in adults across settings (271/1,842; 12 [14])</td>
<td>72.5 (62.9-80.4)</td>
<td>80.1 (71.2-86.2)</td>
<td>10.6 (4.8-23.4)</td>
<td>0.782 (0.689-0.872)</td>
<td>27.5</td>
<td>175.0 (150.0-271.5)</td>
</tr>
<tr>
<td>AKI prediction using plasma/serum NGAL (226/1,039; 9 [9])</td>
<td>73.4 (62.3-82.2)</td>
<td>86.6 (72.0-94.8)</td>
<td>17.9 (6.0-53.7)</td>
<td>0.775 (0.679-0.869)</td>
<td>20.2</td>
<td>179.2 (153.9-199.3)</td>
</tr>
<tr>
<td>AKI prediction using urine NGAL (319/1,783; 14 [14])</td>
<td>77.8 (70.9-83.5)</td>
<td>84.3 (72.8-91.3)</td>
<td>18.6 (7.2-48.4)</td>
<td>0.837 (0.762-0.906)</td>
<td>21.9</td>
<td>193.2 (123.7-405.7)</td>
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<tr>
<td>AKI prediction using research-based assays (242/1,730; 14 [18]</td>
<td>76.9 (69.4-83.1)</td>
<td>83.4 (72.0-90.8)</td>
<td>16.7 (7.1-39.7)</td>
<td>0.732 (0.656-0.830)</td>
<td>31.6</td>
<td>246.4 (88.5-277.2)</td>
</tr>
<tr>
<td>AKI prediction using standardized platforms (245/808; 5 [5])</td>
<td>75.4 (63.8-84.2)</td>
<td>89.3 (81.9-93.9)</td>
<td>25.5 (8.9-72.8)</td>
<td>0.830 (0.741-0.918)</td>
<td>7.0</td>
<td>150.6 (145.0-155.0)</td>
</tr>
<tr>
<td>Initiation of RRT across AKI settings (84/1,948; 9 [10])</td>
<td>76.0 (65.1-84.4)</td>
<td>80.3 (59.5-91.9)</td>
<td>12.9 (4.9-33.9)</td>
<td>0.782 (0.648-0.917)</td>
<td>9.5</td>
<td>278.3 (141.9-381.6)</td>
</tr>
<tr>
<td>In-hospital mortality across AKI settings (88/1,617; 6 [7])</td>
<td>65.0 (51.2-80.8)</td>
<td>82.6 (51.8-95.5)</td>
<td>8.8 (1.9-40.8)</td>
<td>0.706 (0.530-0.747)</td>
<td>10.3</td>
<td>212.0 (121.8-506.7)</td>
</tr>
</tbody>
</table>
Clinical interest of NGAL
Haase M et al meta-analysis Am J Kidney Dis 2009

- NGAL was found a useful early predictor of AKI in all clinical settings
  - much better than creatinine
  - Overall performance AUC-ROC > 0.7 (similar than troponin for MI)
- Urine and serum/plasma NGAL performed similarly well
- NGAL performances improved on standardized analysers vs manual research kits, with cutoff NGAL > 150 ng/mL
- NGAL displayed a prognostic value for RRT (renal replacement therapy) and mortality
- NGAL displayed better performances in pediatric population than in adults
What are the analytical methods available?

- “research-based” assays NGAL Rapid ELISA assay kit, Bioponto, AntibodyShop, Gentofte, Denmark
  - For different biological matrices (urine, serum, plasma)
  - Some home made adaptations with other antibodies or reagents
- Triage kit (Biosite Inc, San Diego, USA)
  - Plasma
- ARCHITECT (Abbott Diagn, Chicago, USA)
  - Urine
- Storage conditions
  - Few data
  - Storage at -20°C for several months may result to degradation up to 40% with a wide variability in the decrease levels
  - Recommendation at -80°C for long period
Conclusions

• Urine or serum NGAL appear a potential useful diagnostic and prognostic tool for Acute Kidney Injury
• Most clinical trials reach similar conclusions of performance (cfr recent Meta-analysis)
• Need for further large and multicenter trials using similar protocols and definitions in various fields, e.g.
  – Elderly patients?
  – Kidney transplantation (quality assessment of transplant organs)?
  – Nephrotoxic agents (aminosides, immunosuppressive drugs,…)
  – Best collection timing in different clinical settings?
• Effect time, T°, urine pH, … on stability?
NGAL, a promising marker to early predict the occurrence of acute tubular necrosis (ATN)

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Stuttgart, October 3rd 2011
Acute Tubular Necrosis (ATN)

- Involves the death of tubular cells, could occur in 10-30% of kidney cadaver grafts (UCL-Brussels)
- ATN is one of the most common causes of AKI and Delayed Graft Function (DGF)
- Requires at least 1 dialysis during the first week
- Tubular cells continually replace themselves
  - A correct diagnostic can lead to return to renal function
- ATN could be from toxic or ischemic origin
  - Renal ischemia due to hypo-perfusion in the donor, prolonged warm and cold ischemia times (>20 h), harvesting conditions, surgical procedures, marginal donors (e.g. NHB donors)
Need to find a ATN marker: NGAL?
Study protocol

• We are lacking of a good and early ATN marker
• Objective: assess the interest of urine/blood NGAL to early predict occurrence of ATN
• Patients population: 54 kidney transplant patients
  – Living related donors (LD as reference population): 11
  – Cadaveric donors (CD): 43
• Collection of urine and blood samples for NGAL, creatinine, cystatin C, GFR and urea determinations
  – Donor: pre-Tx sampling
  – Recipient: time 0, 6, 24, 48 hs post-Tx sampling
Why is it important to predict early ATN?

- Already at the donor level, it may influence exclusion or reallocation of a graft to a patient vs another, based on
  - The quality of the graft available
  - The patient risk factors (age,…)
- Later, after Tx, if the marker predicts a possible risk of ATN, it could help to more closely monitor the patient at risk for delayed graft function
  - Possible blood ion impairment (cardiac arythmia,…)
  - Anticipate availability of dialysis
# Patients population

<table>
<thead>
<tr>
<th></th>
<th>DONORS</th>
<th>RECIPIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD</td>
<td>CD</td>
</tr>
<tr>
<td></td>
<td>11/49</td>
<td>38/49</td>
</tr>
<tr>
<td></td>
<td>(22.4%)</td>
<td>(77.6%)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52.0</td>
<td>57.5</td>
</tr>
<tr>
<td></td>
<td>43.0-65.0</td>
<td>2.0-85.0</td>
</tr>
<tr>
<td>Male</td>
<td>7(63.6%)</td>
<td>22(51.2%)</td>
</tr>
<tr>
<td>Female</td>
<td>4(36.4%)</td>
<td>20(44.4%)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>80.5</td>
<td>75.0</td>
</tr>
<tr>
<td></td>
<td>66.0-98.0</td>
<td>15.0-110.0</td>
</tr>
<tr>
<td>BMI (Kg/cm^2)</td>
<td>26.0</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>20.0-29.0</td>
<td>13.0-38.0</td>
</tr>
</tbody>
</table>
Biomarkers analyses

• Blood samples stored at -70°C until analyses
  – Stability issues well validated
• Urine NGAL and plasma cystatin C reagents were from Abbott Diagnostics on ARCHITECT analyzer
• Urine and plasma creatinine levels were determined with Beckman Coulter reagents
• Plasma NGAL was obtained from Inverness Medical-Alere Ltd (Triage)
• GFR was calculated from the MDRD equation
Results (1)
Ability to differentiate both donor groups

- 24% (13/54) ATN episodes:
  - 0 ATN in LD (0/11)
  - 13 ATN in CD (13/43)

- Significant differences between LD and CD in urine and blood NGAL

- No significant differences between LD and CD for other biomarkers (creatinine, GFR, cystatin C, urea)
Results (2)
Ability to predict ATN at the donor level

- The best predictor for ATN before Tx was urine NGAL (NS)
  - NGAL\textsubscript{urine} med : 9.6 (non ATN) vs 38.6 ng/mL (ATN)
    - $p=0.066$

Supposed to be healthy

Proposed cut-off value
80 ng/mL
Results (3)

Ability to predict ATN at the donor level

- Difficulties to get urine from cadaveric donors from outside
- Trend to prediction from cystatin C (NS)
  - Cystatin C med: 0.83 (non ATN) vs 1.12 mg/L (ATN) p=0.076
- No prediction from creatinine urine or serum, NGAL plasma, GFR

![Box plot and ROC curve showing the prediction of ATN at the donor level.](Image)
Results (4)

Ability to predict ATN at the recipient level (T0h)

• No statistical difference in creatinine$_{serum}$ or urine levels in LD vs CD
• Median NGAL and Cystatin C values were significantly lower at the recipient level at time 0 (just before Tx) in case of LD
  – NGAL$_{urine}$ 155 (LD) vs 850 ng/mL (CD) p <0.01
  – NGAL$_{plasma}$ 487 (LD) vs 656 ng/mL (CD) p <0.05
  – Cystatin C 3.6 (LD) vs 4.7 ng/L (CD) p < 0.001
  – Better patient conditions, shorter waiting list, maintain of a certain residual renal function (preemptive Tx),…in case of LD Tx
Results (5)
Ability to predict ATN at the recipient level (T6h)

- The best predictor for ATN after Tx was NGAL\textsubscript{plasma}
  - NGAL\textsubscript{plasma} med: 428 (non ATN) vs 650 ng/mL (ATN) p < 0.001
Results (6)
Ability to predict ATN at the recipient level (T6h)

- NGAL_{urine} was also able to predict occurrence of ATN
  - NGAL_{urine} med: 244 (non ATN) vs 438 ng/mL (ATN) p = 0.026

![Graph showing NGAL levels for non-ATN and ATN groups]

- Cystatin C, GFR, and creatinine_{serum} but not creatinine_{urine} are also able to predict occurrence of ATN (p ≤ 0.05)
Results (7)
Best time for NGAL sample collection

- Should take into account the clinical interest
  - The earliest, the best: after 3 days relative loss of interest
  - At the donor level
  - After kidney Tx, T6h appears to be the best time to predict ATN based on NGAL_{plasma}

![Graphs showing NGAL levels at different time points (T=0h, T=6h, T=24h, T=48h)](image)

- T= 0h
- T= 6h
- T= 24h
- T= 48h

P<0.05  P<0.01  P<0.05  P<0.05
Results (8)
Interest for $\text{NGAL}_{\text{urine}}$ correction by creatinine?

- No apparent improvement in the prediction performances
  - $\text{NGAL}_{\text{urine}}$ T6 to predict ATN: $p = 0.026$
  - $\text{NGAL}_{\text{urine/creat}}$ T6 to predict ATN: $p = 0.044$