Pro-enkephalin and Renal Disease

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Disclosure

• The presenter has received relevant grants and/or consulting fees from:

Spingotec.
“Tree Trunks Trudy”

- An overweight hypothyroid woman who has deep vein thrombosis undergoes an MRI angiography.
- She is teased in school as having “tree trunk thighs.”
- Creatinine testing is performed. The result is within the normal range.
Renal function testing for radiology

- Iodinated contrast media is used for MRI and interventional radiology but suffers from acute renal failure.
- Gadolinium-based media is thought to be safer?
Acute kidney injury?

• Trudy normally takes a non-selective non-steroidal anti-inflammatory drug for arthritic pain.
• A few days before the procedure, she doubles the dose due to a flare up.
• On the day of the procedure she has AKI *but it is unrecognized by her medical team*
• She undergoes the angiogram.
Ideal characteristics for an AKI biomarker

Bhagwati. Emerging biomarkers of kidney injury

**Biological properties**
- Rapid and increase after AKI
- Sensitive for AKI
- Specific for AKI:
  - Etiology specific
- Correlate with injury severity
- Provide prognostic information

**Physiochemical properties**
- Stable over temperature, pH
- Easily measured in serum/urine
- Inexpensive assay
- No false positive increases

*Applicable over a range of populations*
Creatinine

- Marker of renal function.
- Begins to increase when there is 50% loss of function.
- High inter-individual variation: age, gender, ethnicity, muscle mass, diet, nutritional status.
- Low intra-individual biological variation.
Significant decrease in GFR over baseline. Marker of disease monitoring.

BV for creatinine
Tissue inhibitor of MMP-2 and insulin-like growth factor binding protein 7

**TIMP-2:**
- Inhibitor protein to matrix metalloproteinases, peptidases that degrade extracellular collagen
- Suppresses proliferation of endothelial cells

**IGFBP-7:**
- Regulates the availability of insulin growth factors
- Stimulates cell adhesion

- Both factors are involved with G1 cell-cycle arrest induced by ischemia or sepsis.
- Indicator of AKI = [TIMP-2] x [IGFBP-7]
TIMP-2*IGFBP-7 after Cardiac Surgery
Meersch et al. Plos One 2014;93460
TIMP-2*IGFBP-7 predicting renal recovery
Meersch et al. Plos One 2014;93460
TIMP-2*IGFBP-7 vs clinical parameters
Meersch et al. Plos One 2014;93460

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference Risk Model</th>
<th>New Risk Model (addition of TIMP-2*IGFBP7 to reference risk model)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>p-value</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.12 (0.15–8.61)</td>
<td>0.92</td>
</tr>
<tr>
<td>APACHE III Score</td>
<td>1.74 (1.23–2.69)</td>
<td>0.01</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>10.05 (0.67–150.72)</td>
<td>0.09</td>
</tr>
<tr>
<td>Baseline creatinine</td>
<td>0.06 (0.01–3.074)</td>
<td>0.16</td>
</tr>
<tr>
<td>X-Clamp time</td>
<td>1.01 (0.99–1.04)</td>
<td>0.30</td>
</tr>
<tr>
<td>COPD</td>
<td>40.28 (2.2–737.82)</td>
<td>0.01</td>
</tr>
<tr>
<td>TIMP-2*IGFBP7</td>
<td>Not included in model</td>
<td></td>
</tr>
</tbody>
</table>
Neutrophil Galactinase-Associated Lipocalin (NGAL)

- GAL is involved in innate immunity by sequestering iron that in turn limits bacterial growth
- Found in neutrophils (monomers and dimers), kidneys, prostate, respiratory and alimentary tracts (monomers)
- Low molecular weight (25 kDa) and excreted into urine
Release of NGAL during AKI
Enkephalins

- Met- and Leu-Enkephalin: Endogenous Pentapeptides
- Enkephalins play a major role in a variety of physiological processes
  - Perception of pain
  - Regulation of stress
  - Regulation of cardiovascular function
  - Regulation of bone formation
  - Regulation of immune responses
- Involvement in kidney (dys-)function not yet described
Proenkephalin

• Enkephalin is not stable *in-vivo* and *in-vitro*
  – Not applicable for laboratory routine use
• Proenkephalin (119-159):
• Stable fragment of the precursor molecule

• Plasma proenkephalin is a stable surrogate marker for enkephalin
Enkephalin and receptors expressed in kidney

Enkephalin receptors

Enkephalin

Link of Enkephalin and Enkephalin receptors to kidney
proenkephalin – AKI versus no AKI

Proenkephalin is significantly elevated preoperatively and post-operatively in patients who develop AKI
Pro-enkephalin increases quicker than creatinine in patients developing AKI
Proenkephalin: Association with RIFLE
Marino et al. J Nephrol 2014, epub

<table>
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<tr>
<th>Variable</th>
<th>all (n=101)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
</tr>
<tr>
<td>Gender - male</td>
<td>60%</td>
</tr>
<tr>
<td>Age - median [IQR]</td>
<td>78 [72-83]</td>
</tr>
<tr>
<td><strong>concomitant diseases</strong></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular - yes</td>
<td>26%</td>
</tr>
<tr>
<td>Hypertensive - yes</td>
<td>47%</td>
</tr>
<tr>
<td>Diabetes - yes</td>
<td>35%</td>
</tr>
<tr>
<td>Cancer - yes</td>
<td>13%</td>
</tr>
<tr>
<td><strong>routine laboratory variables</strong></td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance (mL/min) - median [IQR]</td>
<td>48 [23-77]</td>
</tr>
<tr>
<td>PCT (ng/mL) - median [IQR]</td>
<td>2.8 [0.6-10.7]</td>
</tr>
<tr>
<td><strong>other</strong></td>
<td></td>
</tr>
<tr>
<td>APACHE II score (points) - median [IQR]</td>
<td>16 [13-21]</td>
</tr>
<tr>
<td>Final diagnosis - severe sepsis or shock</td>
<td>29%</td>
</tr>
<tr>
<td>Days hospitalized - median [IQR]</td>
<td>5 [2-7]</td>
</tr>
<tr>
<td>Deaths within 7 days after presentation</td>
<td>28%</td>
</tr>
</tbody>
</table>

=> Typical septic ED population: old, many comorbidities
Proenkephalin: Association with RIFLE
Marino et al. J Nephrol 2014, epub

Proenkeph: 99th percentile 80 pmol/L
NGAL: normals < 0.1 ug/mL

Suspected sepsis patients presenting to the ED
Proenkephalin increased in early deaths
Marino et al. J Nephrol 2014, epub

<table>
<thead>
<tr>
<th>Variable</th>
<th>all (n=101)</th>
<th>deaths within 7 days (n=28)</th>
<th>7 day survivor (n=73)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>penKid (pmol/L)</td>
<td>87 [50-205]</td>
<td>209 [77-499]</td>
<td>75 [47-124]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NGAL (µg/mL)</td>
<td>0.6 [0.4-1.2]</td>
<td>1.3 [0.5-2.1]</td>
<td>0.6 [0.3-0.8]</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>48 [23-77]</td>
<td>33 [15-69]</td>
<td>56 [29-81]</td>
<td>0.071</td>
</tr>
</tbody>
</table>

→ penKid is stronger than Creatinine clearance to predict death (which frequently results from kidney failure)
proenkephalin – a highly dynamic biomarker

Patient 1 died

Patient 2 survived

Patient 3 died

Patient 4 survived

penKid [pmol/L]

T I M E

0H 12H 24H 48H

normal range

penKid [pmol/L]

0H 12H 24H 48H

normal range

penKid [pmol/L]

0H 12H 24H 48H

normal range

penKid [pmol/L]

0H 12H 24H 48H

normal range

Patient 1 died

Patient 2 survived

Patient 3 died

Patient 4 survived
Kidney dysfunction detected by penenkephalin predicts 2-y mortality in AMI patients

**Acute myocardial infarction**

Ng (UK)

n = 1,141

Event free portion for patients with penKid levels **above** or **below** the cut-off (99th percentile of normal range)

Ga-induced nephrogenic systemic fibrosis

- Fibrosis of the skin, eyes, joints and organs.
- NSF occurs in patients with eGFR <60 mL/min or creatinine >3 mg/dL.
“Tree Trunks Trudy”

- AKI occurs in 1-5% of patients on NSAIDs.
- 70 million prescriptions, 2.5 million AKIs/y.
- Trudy had AKI and developed systemic fibrosis.
- Blood from her creatinine test was saved and tested for AKI biomarkers. They were positive.
- Angiography should have been delayed.
- The Hidden Assassin: When Clinical Lab Tests Go Awry.