Novel Biomarkers for Liver Injury and Hepatorenal Syndrome

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DISCLOSURES: Consultant to Sanofi-Aventis, Exhalenz, Pfizer, Salix, Orphan Therapeutics, Gilead, Astellas, Ikaria

Renal dysfunction in patients with cirrhosis and end-stage liver disease (n=19261)

Mean GFR: 14 ml/min (severe)
Mean GFR: 30 ml/min (moderate)
Mean GFR: 56 ml/min (mild)
Mean GFR: 118 ml/min (normal)


Spectrum of renal disease in cirrhosis

Cirrhosis alone
- Pre-renal:
  - Hypovolemia
  - Hepatorenal
- Intra-renal:
  - Acute tubular necrosis
  - Drug-induced
- Post-renal:
  - Obstructive uropathy

Cirrhosis + chronic kidney disease
- HCV: MPGN
- HBP: membranous GN
- Infection related renal dysfunction
  - Hypovolemia
  - ATN
  - HRS
  - Iatrogenic
Renal Failure in Hospitalized Cirrhotics

- Cirrhosis
  - Hospitalized
  - Chronic Renal Failure 1%
  - Acute Renal Failure 19%
  - Pre-Renal 68%
  - Intra-Renal (ATN) 32%
  - Post-Renal <1%

  - Volume Responsive 66%
  - Not Volume Responsive
    - HRS Type 1 25%
    - HRS Type 2 9%


Differential Diagnosis Between HRS and ATN

<table>
<thead>
<tr>
<th></th>
<th>HRS</th>
<th>ATN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent shock</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Response to volume challenge</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Urine sodium (mmol/L)</td>
<td>&lt;20</td>
<td>&gt;40</td>
</tr>
<tr>
<td>FENa</td>
<td>&lt;1</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg)</td>
<td>&lt;500</td>
<td>&gt;350</td>
</tr>
<tr>
<td>Granular casts</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

FENa, fractional excretion of sodium.

Combinations of abnormalities in urine of subjects with cirrhosis and renal dysfunction

- Creatinine > 1.5 mg/dl
- Proteinuria > 0.5 gm/day
- Hematuria

N= 65

Trawalle et al, Liver International, Liver Int. 2010 May;30(5):725-32
CKD is commonly present in patients with cirrhosis admitted with AKI

Belcher et al, Hepatology, in press, 2012

Renal Biopsy findings in 44 subjects with cirrhosis, renal failure with non-classic urine findings

<table>
<thead>
<tr>
<th>Histologic finding</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular pathology</td>
<td>31 (71)</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>20 (45)</td>
</tr>
<tr>
<td>MPGN</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Diabetic glomerulosclerosis</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Focal segmental glomerulosclerosis</td>
<td>4 (9)</td>
</tr>
<tr>
<td>ATN</td>
<td>18 (41)</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>12 (27)</td>
</tr>
</tbody>
</table>

Wadei et al, Am J Transplantation, 2006; 8:2618-2626

• ATN missed: renal support denied to patient
• Need for simultaneous liver kidney transplant (SLK)
• Incorrect assessment of treatments for HRS
• HRS missed: vasoconstrictors not given

Why do we need biomarkers for AKI in patients with cirrhosis

• There are many causes of AKI in the subject with cirrhosis.
• Infections play a major role in this process and can cause AKI via multiple mechanisms
• Current methods of assessment are based on organ failure and are suboptimal for guidance of management
• Incorrect classification and management has major consequences for the patient.
Properties of an ideal biomarker

- Highly sensitive (for early diagnosis)
- Highly specific (to assess treatment effect)
- Reflects abnormal physiology
- Actionable:
  - basis for biomarker-guided therapy
  - prognostic value is greatest if it guides intervention and outcomes

Cruz et al, Contrib Nephrol, 2013, 182:45-64

AKI is cirrhosis may involve functional and structural changes

Cruz et al, Contrib Nephrol, 2013, 182:45-64

Differential diagnosis of AKI and how biomarkers could be used

Endre et al, Contrib Nephrol 2013, 182: 30-44
Biomarkers in development

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Functional</th>
<th>Oxidative stress</th>
<th>Structural Cell injury</th>
<th>Immune response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure</td>
<td>Cystatin-C</td>
<td>Glycation Products</td>
<td>Albuminuria</td>
<td>N-GAL</td>
</tr>
<tr>
<td>Urine output</td>
<td>Capillary pressure/flow markers</td>
<td>Lipid Oxidation</td>
<td>Podocyte Injury</td>
<td>IL-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VCAM-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CXC</td>
</tr>
</tbody>
</table>

Tesch G. Nephronology, 2010; 15:609-616

Can we predict AKI or detect it very early?

Progression of renal insult to renal shutdown

- Normal Renal function
- Insult and injury
- Organ failure
- Creatinine normal
- Rising Creatinine (AKI)

Organ Death (RRT) = renal replacement therapy
Utility of NGAL to predict AKI

N=95

Portal et al, LIVER TRANSPLANTATION 16:1257-1266, 2010

Predicting AKI in cirrhotic subjects


Relationship of NGAL and protein:creatinine ratio

Endothelin in HRS


Distinguishing ATN from functional AKI (pre-renal or HRS)

Summary Statistics for Urine Biomarkers by Adjudicated Diagnosis

<table>
<thead>
<tr>
<th>Marker</th>
<th>PRA N=19</th>
<th>HRS n=16</th>
<th>ATN n=39</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular injury:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGAL (ng/ml)</td>
<td>78</td>
<td>115</td>
<td>565</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-18 (pg/ml)</td>
<td>46</td>
<td>37</td>
<td>124</td>
<td>0.06</td>
</tr>
<tr>
<td>KIM-1 (ng/ml)</td>
<td>4.6</td>
<td>7.6</td>
<td>8.4</td>
<td>0.13</td>
</tr>
<tr>
<td>L-FABP (ng/ml)</td>
<td>10</td>
<td>14</td>
<td>27</td>
<td>0.05</td>
</tr>
<tr>
<td>Tubular function:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FENa (%)</td>
<td>0.28</td>
<td>0.1</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Glomerular injury:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>21</td>
<td>24</td>
<td>92</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Belcher et al, 2013- (YALE-VCU) ms under review
Utility of biomarkers for separation of ATN from functional AKI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimal cutpoint</th>
<th>AUC</th>
<th>sensitivity</th>
<th>specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGAL (ng/ml)</td>
<td>466</td>
<td>0.75</td>
<td>0.56</td>
<td>0.89</td>
</tr>
<tr>
<td>IL-18 (pg/ml)</td>
<td>126</td>
<td>0.66</td>
<td>0.49</td>
<td>0.83</td>
</tr>
<tr>
<td>KIM-1 (ng/ml)</td>
<td>15.4</td>
<td>0.62</td>
<td>0.38</td>
<td>0.91</td>
</tr>
<tr>
<td>L-FABP (ng/ml)</td>
<td>25</td>
<td>0.66</td>
<td>0.54</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Belcher et al, 2013- (YALE-VCU) ms under review

Identification of ATN with a multiple biomarker panel

Belcher et al, 2013- (YALE-VCU) ms under review

Percentage with number of biomarkers above cutoff

Belcher et al, 2013- (YALE-VCU) ms under review
NGAL values as a guide for differential diagnosis

<table>
<thead>
<tr>
<th>Author</th>
<th>Pre-renal</th>
<th>HRS</th>
<th>ATN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verna</td>
<td>20</td>
<td>105</td>
<td>325</td>
</tr>
<tr>
<td>Fagundes</td>
<td>30</td>
<td>76</td>
<td>417</td>
</tr>
<tr>
<td>Belcher</td>
<td>78</td>
<td>111</td>
<td>462</td>
</tr>
</tbody>
</table>

NGAL values in ng/ml

Biomarkers to assess progression and long-term outcomes

Progression of AKIN is a major determinant of death

Belcher et al, Hepatology, 2012, in press
New biomarkers: klotho


Potential role of KIM1 and NGAL in progression from AKI to CKD


Summary and conclusions

• There is a clear need for better biomarkers for prediction, early detection, differential diagnosis, treatment guidance and prognosis of AKI in cirrhosis
• While NGAL and other markers are promising, much more work needs to be done before these can be used in routine practice