Potential biomarkers for chronic kidney disease

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Background

Course of Chronic kidney disease

Increased cell death and counterregulation

Source: Kidney Int © 2007 International Society of Nephrology
Cytokeratin-18 as biomarker for CKD?

HSPs as biomarker for CKD?
Cytokeratin-18 as biomarker for CKD?

Cytokeratin 18

Intermediate filament protein
ubiquitous expressed by epithelial and parenchymal cells
Background – CK18

Total CK-18

Necrosis

total CK-18↑

Serum levels

http://www.hg-kempen.de/bilder/Blutroehrchen.jpg
Background – CK18

Apoptosis
- total CK-18 cleaved by caspases 3, 7 and 9

Caspase 7

Serum levels

Caspase cleaved CK-18↑

Necrosis

Total CK-18

http://asterix.cs.gsu.edu/~weber/caspase.html

http://www.hg-kempen.de/bilder/Blutroehrchen.jpg
Background – CK18

CK -18 as biomarker for CKD?
comparison

CK-18 as biomarker for CKD?

Comparison

CK - 18

48 kDa
430 AA
cck 18, 30 AA
Marker for cell death
Elevated in urine of patients with CKD?

Albumin

66 kDa
585 AA
Mikroalbuminurie: 7%

Results – CK18

total CK-18 levels
in 120 patients + 20 controls

Serum

![Diagram showing total CK18 levels in different stages of CKD and healthy controls.](image)
Results – CK18
total CK-18 levels
in 120 patients + 20 controls
Results – CK18
total CK -18 levels
in 120 patients + 20 controls
Results – CK18
ROC curve analysis
total CK-18 levels

Serum total CK18 - Stage 3 or higher

AUC 0.749
(0.645-0.853)
p<0.001

Serum total CK18 - Stage 5

AUC 0.690
(0.584-0.796)
p<0.001

Urine total CK18 - Stage 5

AUC 0.799
(0.695-0.903)
p<0.001

Albumin - Stage 5

AUC 0.727
(0.556-0.898)
p=0.015
Results – CK18

Findings in patients with CKD - summary
Results – CK18

Findings in patients with CKD - summary

• caspase cleaved CK-18 NOT elevated in serum or urine
Findings in patients with CKD - summary

- caspase cleaved CK-18 NOT elevated in serum or urine

- total CK-18 significant elevated in serum and urine
Findings in patients with CKD - summary

- caspase cleaved CK-18 NOT elevated in serum or urine

- total CK-18 significant elevated in serum and urine

→ indication for increased necrotic cell death
Heat shock proteins

as biomarkers for CKD?

Background – HSP

Heat shock proteins

- Cellular defense mechanisms
- Inhibit apoptotic pathways
Background – HSP

Heat shock proteins

- Cellular defense mechanisms
- Inhibit apoptotic pathways

**HSP27:** 27kDa
- Inhibits caspase activation
- Anti-oxidant qualities
Background – HSP

Heat shock proteins

- Cellular defense mechanisms
- Inhibit apoptotic pathways

**HSP27**: 27kDa
- Inhibits caspase activation
- Anti-oxidant qualities

**HSP70**: 70kDa
- Inhibits intrinsic and extrinsic pathways
- Inhibits cell damage by urea *in vitro*
Heat shock proteins

- Cellular defense mechanisms
- Inhibit apoptotic pathways

**HSP27**: 27kDa
- Inhibits caspase activation
- Anti-oxidant qualities

**HSP70**: 70kDa
- Inhibits intrinsic and extrinsic pathways
- Inhibits cell damage by urea *in vitro*
- Unchanged serum levels in children with CKD (Musial et al. 2010)
- Decreased expression in blood monocytes of adults with CKD (Marzec et al. 2009)
Results – HSP

HSP70 levels

**Urine** (119 patients, 23 controls)

![Graph showing HSP levels in urine across different stages of CKD]

- Healthy: ns
- CKD 1: p = 0.0227
- CKD 2: p = 0.002
- CKD 3: ns
- CKD 4: ns
- CKD 5: ns

**Serum levels:** technical limitations, data for remaining 42 patients not significant

**Fractional excretion:** significant elevated in stage 5 (p = 0.0027)
Results – HSP

HSP27 levels
in 119 patients + 23 controls
Results – HSP

HSP27 levels in 119 patients + 23 controls

**Serum HSP-27, pg/ml**
- Healthy: ns
- CKD 1: ns
- CKD 2: p < 0.001
- CKD 3: p < 0.001
- CKD 4: p < 0.001
- CKD 5: ns

**Urine HSP-27, pg/ml**
- Healthy: ns
- CKD 1: ns
- CKD 2: ns
- CKD 3: ns
- CKD 4: p < 0.001
- CKD 5: ns
Results – HSP

HSP27 levels
in 119 patients + 23 controls
Results – HSP

ROC curve analysis

HSP27

Serum HSP27 - Stage 3 or greater

AUC 0.9191
(0.8626 - 0.9756)
p < 0.0001

Fractional HSP27 excretion - Stage 1 or higher

AUC 0.8159
(0.7285 - 0.9034)
p < 0.0001
Results – Summary

- total CK-18 significant *elevated* in serum and urine
  → indication for increased necrotic cell death

- Heat shock proteins significant *elevated* in serum and urine
  → counterregulation to increased systemic stress levels
Potential biomarkers for chronic kidney disease
<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>CKD 1</th>
<th>CKD 2</th>
<th>CKD 3</th>
<th>CKD 4</th>
<th>CKD 5</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>59 (19–88)</td>
<td>36 (19–61)</td>
<td>50 (19–80)</td>
<td>63 (23–78)</td>
<td>61 (29–88)</td>
<td>65 (20–81)</td>
<td>30 (21–67)</td>
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<tr>
<td><strong>Gender (male/female)</strong></td>
<td>67/53</td>
<td>7/3</td>
<td>9/14</td>
<td>27/14</td>
<td>15/12</td>
<td>9/10</td>
<td>15/8</td>
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<tr>
<td><strong>Kidney disease</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Glomerulonephritis</td>
<td>30</td>
<td>4</td>
<td>7</td>
<td>13</td>
<td>7</td>
<td>3</td>
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<tr>
<td>Polycystic kidney disease</td>
<td>16</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td></td>
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<tr>
<td>Vascular nephropathy</td>
<td>26</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td></td>
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<tr>
<td>Interstitial nephropathy</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
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<tr>
<td>Bilateral nephrectomy</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Urine stasis</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>Unknown</td>
<td>36</td>
<td>2</td>
<td>5</td>
<td>11</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>Serum creatinine (mg dL⁻¹)</strong></td>
<td>1.84 (0.72–6.88)</td>
<td>0.90 (0.72–1.03)</td>
<td>0.99 (0.77–1.52)</td>
<td>1.62 (1.02–2.34)</td>
<td>2.73 (2.04–3.89)</td>
<td>5.00 (3.47–6.88)</td>
<td>0.99 (0.77–1.20)</td>
</tr>
<tr>
<td><strong>Blood urea nitrogen (mg dL⁻¹)</strong></td>
<td>31.9 (7.1–91.2)</td>
<td>12.6 (7.5–17.6)</td>
<td>13.9 (7.1–33.4)</td>
<td>30.5 (11.6–64.1)</td>
<td>51.2 (23.8–91.2)</td>
<td>63.3 (31.9–87.3)</td>
<td>13.1 (8.2–20)</td>
</tr>
<tr>
<td><strong>Urine creatinine (mg dL⁻¹)</strong></td>
<td>69.2 (12.7–294.5)</td>
<td>69.1 (22.7–252.9)</td>
<td>77.3 (14.8–243.5)</td>
<td>78 (12.7–294.5)</td>
<td>923 (291–2464)</td>
<td>854 (267–1370)</td>
<td>647 (273–1381)</td>
</tr>
<tr>
<td><strong>Urine protein (g L⁻¹)</strong></td>
<td>0.2 (&lt;0.05–6.94)</td>
<td>0.06 (&lt;0.05–0.21)</td>
<td>0.1 (&lt;0.05–4.45)</td>
<td>0.1 (&lt;0.05–2.79)</td>
<td>0.29 (&lt;0.05–3.46)</td>
<td>1.07 (0.05–6.94)</td>
<td></td>
</tr>
<tr>
<td><strong>GOT (U L⁻¹)</strong></td>
<td>22 (9–88)</td>
<td>24.5 (15–88)</td>
<td>22 (12–62)</td>
<td>26 (14–57)</td>
<td>22 (9–60)</td>
<td>18 (10–69)</td>
<td></td>
</tr>
<tr>
<td><strong>GPT (U L⁻¹)</strong></td>
<td>21 (&lt;3–98)</td>
<td>29.5 (11–75)</td>
<td>20 (11–72)</td>
<td>25 (9–63)</td>
<td>17 (6–51)</td>
<td>15 (&lt;3–98)</td>
<td></td>
</tr>
<tr>
<td><strong>Gamma-GT (U L⁻¹)</strong></td>
<td>28.5 (9–561)</td>
<td>38.5 (13–561)</td>
<td>25 (10–134)</td>
<td>36 (9–472)</td>
<td>27 (10–303)</td>
<td>24 (13–346)</td>
<td></td>
</tr>
</tbody>
</table>

Date are given as median with range; CKD, chronic kidney disease.
Fig. 2. Panel A: Total urine protein and total urine CK-18 correlated significantly using Spearman’s rank correlation coefficient (Spearman’s $r = 0.5178$, $p < 0.0001$, 119 pairs). The X as well as the Y-axis is given as log scale. Panel B: Urine albumin and total urine CK-18 correlated significantly using Spearman’s rank correlation coefficient (Spearman’s $r = 0.4377$, $p < 0.0001$, 119 pairs). The X as well as the Y-axis is given as log scale.
Correlation of HSP serum and urine levels with clinical and kidney function parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HSP27 serum</th>
<th>HSP27 urine</th>
<th>HSP70 urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (119 pairs)</td>
<td>r = 0.3625, p &lt;0.0001</td>
<td>r = -0.1249, p = 0.1760</td>
<td>r = -0.04412, p = 0.6338</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate (119 pairs)</td>
<td>r = -0.5414, p &lt; 0.0001</td>
<td>r = -0.01213, p = 0.1887</td>
<td>r = -0.1770, p = 0.0541</td>
</tr>
<tr>
<td>Total urine protein (118 pairs)</td>
<td>r = 0.3599, p &lt; 0.0001</td>
<td>r = 0.2364, p = 0.0100</td>
<td>r = 0.2187, p = 0.0174</td>
</tr>
<tr>
<td>Urine albumin (92 pairs)</td>
<td>r = 0.3244, p = 0.0016</td>
<td>r = 0.1935, p = 0.0645</td>
<td>r = 0.1055, p = 0.3170</td>
</tr>
<tr>
<td>Creatinine clearance (33 pairs)</td>
<td>r = -0.595, p = 0.0003</td>
<td>r = -0.2697, p = 0.129</td>
<td>r = -0.2635, p = 0.1385</td>
</tr>
<tr>
<td>CRP (118 pairs)</td>
<td>r = 0.2804, p = 0.0021</td>
<td>r = 0.04044, p = 0.6637</td>
<td>r = 0.01164, p = 0.9005</td>
</tr>
</tbody>
</table>