Multiplex Measurement of Human Kidney Biomarkers in Serum Using the Magnetic Luminex® Performance Assay

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Abstract

The kidneys play an important role in organellar homeostasis by regulating osmolality and blood pressure, aiding in the reabsorption of water and nutrients, secreting hormones, and excreting waste. Serum biomarkers of acute kidney injury (AKI) are an important experimental tool for drug development. Historically, renal function has been evaluated by measuring serum creatinine and blood urea nitrogen (BUN). Recent advances in renal biomarkers have included the development of sensitive ELISA kits with a slope of 0.9–1.1 and R2 > 0.9. ELISA kits have a limited number of samples, and serum biomarkers are still required for a complete panel.

Introduction

The kidneys are a vital organ with many important roles in maintaining organellar homeostasis. During the execution of these roles and the reabsorption of water and nutrients, the kidneys are especially vulnerable to the effects of toxic compounds including drugs and metabolites. Impaired kidney function can be the result of either acute kidney injury (AKI) or chronic kidney disease (CKD). AKI can be caused by toxins, trauma, or drug toxicity while CKD is a complication of diabetes mellitus, hypertension, polycystic kidney disease, and glomerulonephritis. These disorders are associated with a reduction in kidney function, and changes in these parameters can be used to assess kidney function. The development of renal markers can be used to assess kidney function in the clinic.

Methods

Serum samples from ten apparently healthy individuals were evaluated by measuring serum creatinine and blood urea nitrogen levels. Recently, more sensitive kidney biomarkers have been developed, including the Luminex Performance Human Kidney Biomarker Assay. This assay was run according to kit instructions using a protocol to adapt this kit for NIR fluorescence detection using a LI-COR Odyssey® Infrared Imaging System.

Discussion and Conclusions

Using the Luminex Performance Human Kidney Biomarker Assay, we detected significant differences in Cystatin C, Lipocalin-2, Osteopontin, and/or TFF3 concentrations between AKI, CKD, and apparently healthy samples. Differences in the AKI group compared to the healthy group for Lipocalin-2, Osteopontin, Fetuin A, and Cystatin C. We observed statistically significant differences in the AKI group compared to the healthy group for Lipocalin-2, Osteopontin, Fetuin A, and Cystatin C. We also observed statistically significant differences in the AKI group compared to the healthy group for Lipocalin-2, Osteopontin, Fetuin A, and Cystatin C.

Table 1. Performance Characteristics of the Human Kidney Biomarker Panel.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Catalog #</th>
<th>Sensitivity (pg/mL)</th>
<th>High Standard (pg/mL)</th>
<th>Interassay Precision (CV)</th>
<th>Linearity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C</td>
<td>LHK1586</td>
<td>67</td>
<td>10.500</td>
<td>0.9%</td>
<td>90-110%</td>
</tr>
<tr>
<td>Osteopontin (OPN)</td>
<td>LHK1184</td>
<td>664</td>
<td>3,300,000</td>
<td>≤ 16.7%</td>
<td>74-93%</td>
</tr>
<tr>
<td>Fetuin A/AHSG</td>
<td>LHK4407</td>
<td>11</td>
<td>11,000</td>
<td>≤ 10.9%</td>
<td>83-106%</td>
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<tr>
<td>Lipocalin-2/NGAL</td>
<td>LHK266</td>
<td>0.7</td>
<td>3,110</td>
<td>≤ 16.8%</td>
<td>98-129%</td>
</tr>
<tr>
<td>TIM-1/KIM-1/HAVCR</td>
<td>LHK1433</td>
<td>247</td>
<td>295,500</td>
<td>≤ 10.2%</td>
<td>73-110%</td>
</tr>
<tr>
<td>RBP4</td>
<td>LHK1750</td>
<td>16</td>
<td>100,000</td>
<td>≤ 10.5%</td>
<td>92-110%</td>
</tr>
</tbody>
</table>

Figure 1. Detection of relative differences in 38 proteins using the Proteome Profiler Kidney Biomarker Array Kit. Duplicate spots corresponding to RBPI, Cystatin C, Lipocalin-2, and TF3 are indicated. (A) Samples from apparently healthy individuals. (B) Renal failure samples.

Figure 2. Measurement of kidney biomarkers in serum using the Luminex Kidney Biomarker Magnetic Assay Panel. Mean and SD of results for ten samples in each group are shown. CRCL10/IP-10 and TIM-1/KIM-1/HAVCR were non-detectable in all samples tested.

References