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

M. Anderson, M. Rynning, R. Fuerstenberg, G. Wegner, and J. Schmidt. | R&D Systems, Minneapolis, MN.

D374 1012.2

Abstract

The kidneys play an important role in organismal homeostasis by regulating osmolality and blood pressure, aiding in the reabsorption of water and nutrients, excreting wastes, and secreting hormones. Renal function is also important in the metabolism and excretion of drugs; therefore, analyzing nephrotoxicity using renal biomarkers is an important experimental step during drug development. Historically, renal function has been evaluated by measuring serum creatinine and blood urea nitrogen levels. Recently, more sensitive kidney biomarkers have been identified including TIM-1/KIM-1/HAVCR, Lipocalin-2/NGAL, Osteopontin, Cystatin C, Clusterin, CXCL10/IP-10, RBP-4, Fetuin A/AHSG, and TFF3. Serum samples from 10 healthy individuals, 10 acute kidney injury (AKI) individuals, and 10 individuals diagnosed with renal failure were evaluated using the Human Kidney Biomarker Magnetic Luminex Performance Assay. We observed statistically significant 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 renal failure group compared to the healthy group for TFF3, Lipocalin-2, Clusterin, Fetuin A, and Cystatin C. We also observed statistically significant differences in the AKI group compared to the renal failure group for the following biomarkers: TFF3, Fetuin A, and Cystatin C.

The Human Kidney Biomarker Magnetic Luminex Performance Assay from R&D Systems, Inc. is designed for simultaneous quantitative determination of biomarkers in serum, plasma, or urine.

Introduction

The kidneys are a vital organ with multiple important roles in maintaining organismal homeostasis. During the excretion of wastes 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 trauma, sepsis, or drug toxicity, while CKD may be a complication of diabetes mellitus, severe hypertension, autoimmune diseases or other chronic conditions. Assessment of kidney function has historically relied on measurements of blood pressure, serum creatinine, blood urea nitrogen (BUN), protein-to-creatinine ratio in urine, and urine sediment, as well as changes in glomerular filtration rate (GFR). These assessments are not very sensitive, may be delayed in AKI, and may not detect all types of chronic kidney damage. Furthermore, in experimental animals or in humans, substantial kidney damage may occur without a measurable change in GFR.

The identification of biomarkers that are specific to kidney function has been a recent research focus. Changes in these protein biomarkers can be used in clinical or preclinical studies to assess AKI due to nephrotoxic drugs, ischemia, sepsis, or renal transplantation. Specific renal markers can also be used to assess kidney development in embryogenesis. Measurement of multiple kidney biomarkers can be used to assess kidney function contextually.

The Luminex Performance Human Kidney Biomarker Panel is a multiplex immunoassay for the simultaneous measurement of nine protein biomarkers: Clusterin, CXCL10/IP-10, Cystatin C, Fetuin A/AHSG, Lipocalin-2/NGAL, Osteopontin, RBP4, TFF3, and TIM-1/KIM-1/HAVCR in serum, plasma, and urine samples. The base kit can be used with any combination of the nine analyte-specific bead sets for greater flexibility in experimental design. Using the complete panel, we evaluated serum samples from 10 individuals diagnosed with AKI, 10 with renal failure, and 10 apparently healthy individuals.

Methods

Serum samples from individuals diagnosed with AKI or renal failure were purchased from Bioreclamation. Additional serum samples were obtained from apparently healthy in-house donors; no medical information was available.

Serum samples from two apparently healthy individuals and two individuals with renal failure were screened using the Proteome Profiler™ Human Kidney Biomarker Array Kit (R&D Systems, Catalog # ARY019). The assay was run according to kit instructions using a protocol to adapt this kit for NIR fluorescence detection using the LI-COR Odyssey® Infrared Imaging System.

Serum samples from ten apparently healthy individuals, ten individuals with AKI, and ten individuals diagnosed with renal failure were tested using the Luminex Performance Human Kidney Biomarker Magnetic Assay Kit (R&D Systems, Catalog # LHK000). Samples were diluted 1:10 and 1:4000 and the assay was run according to kit instructions.

Discussion and Conclusions

Using the Proteome Profiler Human Kidney Biomarker Array Kit, we detected differences in Cystatin C, Lipocalin-2, RBP4, and TFF3 in serum from individuals diagnosed with CKD compared to apparently healthy donors. As a result of this screen, we tested a larger number of samples with the Luminex Performance Human Kidney Biomarker Magnetic Assay Kit to simultaneously measure concentrations of 9 different protein biomarkers. Statistically significant differences were seen in Clusterin, Cystatin C, Fetuin A, Lipocalin-2, Osteopontin, and/or TFF3 concentrations between AKI, CKD, and apparently healthy samples. Differences in RBP4 were not significant.

The results demonstrate that the Luminex Performance Human Kidney Biomarker Magnetic Assay Kit is a reliable and efficient tool for drug toxicology studies as it can simultaneously assess the levels of nine kidney biomarkers in a single sample.

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

Analyte	Catalog #	Sensitivity (pg/mL)	High Standard (pg/mL)	Inter-assay Precision (CV)	Linearity
Clusterin	LHK2937	64	483,800	≤ 13.5%	90-97%
Cystatin C	LHK1196	57	16,500	≤ 9.4%	80-101%
CXCL10/IP-10	LHK266	0.7	3,110	≤ 16.8%	98-129%
Fetuin A/AHSG	LHK1184	664	3,300,000	≤ 16.7%	74-93%
Lipocalin-2/NGAL	LHK1757	63	36,100	≤ 13.6%	99-115%
Osteopontin (OPN)	LHK1433	247	295,500	≤ 10.2%	73-110%
RBP4	LHK3378	124	47,400	≤ 15.4%	86-103%
TFF3	LHK4407	11	11,000	≤ 10.9%	83-106%
TIM-1/KIM-1/HAVCR	LHK1750	16	116,000	≤ 10.5%	92-110%

Assays in this panel are correlated to the respective Quantikine® ELISA kits with a slope of 0.9–1.1 and $R^2 > 0.9$.

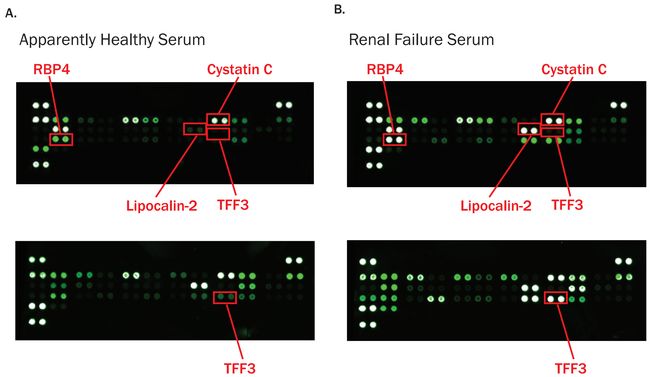

Figure 1. Detection of relative differences in 38 proteins using the Proteome Profiler Kidney Biomarker Array Kit. Duplicate spots corresponding to RBP4, Cystatin C, Lipocalin-2, and TFF3 are indicated. (A) Samples from apparently healthy individuals. (B) Renal failure samples.

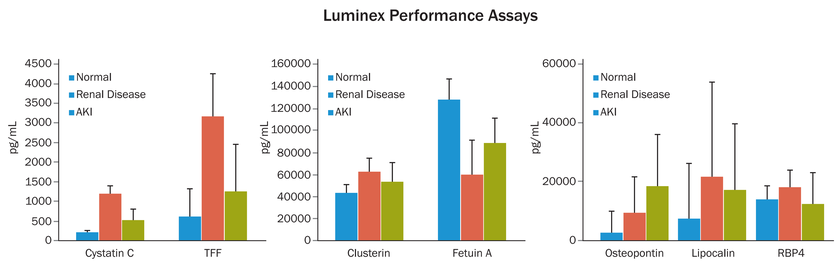

Figure 2. Measurement of kidney biomarkers in serum using the Luminex Kidney Biomarker Magnetic Panel. Mean and SD of results for ten samples in each group are shown. CXCL10/IP-10 and TIM-1/KIM-1/HAVCR were non-detectable in all samples tested.

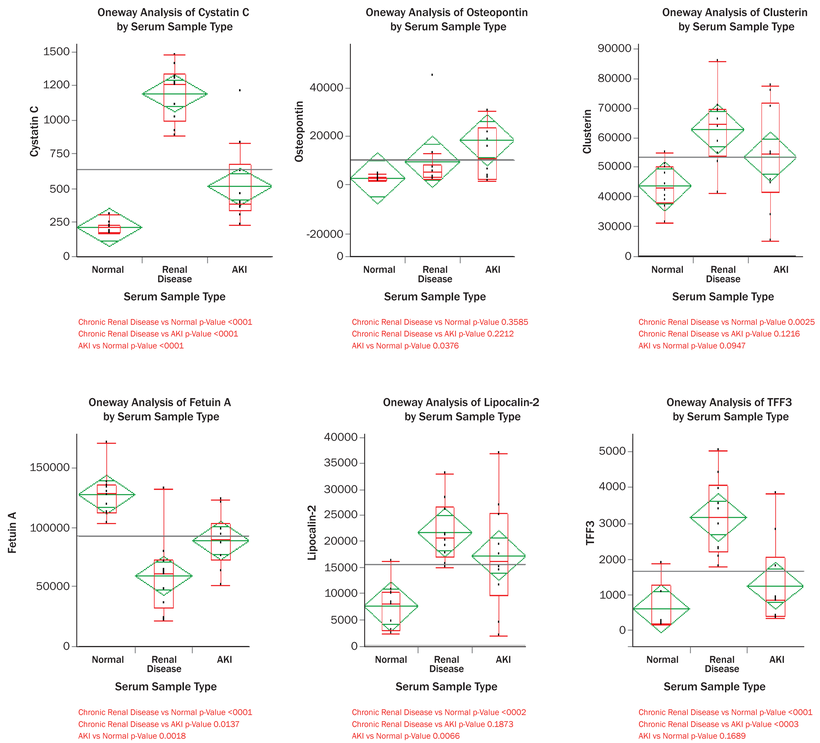

Figure 3. Comparison of kidney biomarker concentrations in serum from AKI, CKD individuals, and apparently healthy donors. Statistically significant differences in Clusterin, Cystatin C, Fetuin A, Lipocalin-2, and TFF3 were observed between CKD and healthy donors, in Cystatin C, Fetuin A, Lipocalin-2, and Osteopontin between AKI and healthy donors, and in Cystatin C, Fetuin A, and TFF3 between AKI and CKD donors. The line across each diamond represents the group mean. The vertical span of each diamond represents the 95% confidence interval for each group.

References

1. Mehta, R.L. *et al.* (2004) Kidney Int. **66**:1613.
2. Ozer, J.S. *et al.* (2010) Nat. Biotech. **28**:486.
3. Bonventre, J.V. *et al.* (2010) Nat. Biotech. **28**:436.
4. Goodsaid, F.M. *et al.* (2009) Clin. Pharmacol. Ther. **86**:490.
5. Vaidya, V.S. *et al.* (2010) Nat. Biotech. **28**:478.
6. Dieterle, F. *et al.* (2010) Nat. Biotech. **28**:455.