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Aptamer-Based Proteomics Identifies Mortality-Associated Serum Biomarkers in Dialysis-Dependent AKI Patients

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Introduction: Currently, no effective therapies exist to reduce the high mortality associated with dialysis-dependent acute kidney injury (AKI-D). Serum biomarkers may be useful in understanding the pathophysiological processes involved with AKI and the severity of injury, and point to novel therapeutic targets.

Methods: Study day 1 serum samples from 100 patients and day 8 samples from 107 patients enrolled in the Veteran's Affairs/National Institutes of Health Acute Renal Failure Trial Network study were analyzed by the slow off-rate modified aptamers scan proteomic platform to profile 1305 proteins in each sample. Patients in each cohort were classified into tertiles based on baseline biomarker measurements. Cox regression analyses were performed to examine the relationships between serum levels of each biomarker and mortality.

Results: Changes in the serum levels of 54 proteins, 33 of which increased and 21 of which decreased, were detected when comparing samples of patients who died in the first 8 days versus patients who survived >8 days. Among the 33 proteins that increased, higher serum levels of fibroblast growth factor-23 (FGF23), tissue plasminogen activator (tPA), neutrophil collagenase (matrix metalloproteinase-8), and soluble urokinase plasminogen activator receptor, when stratified by tertiles, were associated with higher mortality. The association with mortality persisted for each of these proteins after adjusting for other potential risk factors, including age, sex, cardiovascular sequential organ failure assessment score, congestive heart failure, and presence of diabetes. Upper tertile levels of FGF23, tPA, and interleukin-6 on day 8 were associated with increased mortality; however, FGF23 barely lost significance after multivariable adjustment.

Conclusions: Our results underscore an emerging proteomics tool capable of identifying low-abundance serum proteins important not only in the pathogenesis of AKI-D, but which is also helpful in discriminating AKI-D patients with high mortality.

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Acute kidney injury requiring dialysis (AKI-D), one of the most serious complications for hospitalized patients, is associated with prolonged length of stay, mortality, and progressive chronic kidney disease (CKD) among survivors.[1–4] Better understanding of the pathogenesis of AKI-D and risk factors associated with mortality is needed. Previous studies have examined the role of urine and serum biomarkers to predict AKI severity. The Biologic Markers of Recovery for the Kidney study conducted in AKI-D patients found that decreasing urine levels of hepatocyte growth factor and neutrophil gelatinase–associated lipocalin predicted recovery.[5–7] In this study, higher serum levels of tumor necrosis factor receptor 1 and interleukin (IL)-8 were associated with reduced dialysis-free survival at 60 days.[6,7] These studies suggested that serum AKI biomarkers could provide additional prognostic

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information, although they might not have established a direct relationship between the degree of kidney injury and increased mortality.[8]

Mass spectrometry (MS) and affinity multiplexing assays have been used for proteomic biomarker discovery. MS-based proteome profiling is ideal for discovery of novel biomarkers but lacks throughput in the validation phase when hundreds to thousands of samples require analysis.[9] Affinity proteome profiling with high multiplexing capabilities has emerged as a more efficient method for biomarker studies. Affinity-based protein profiling uses antibody bead arrays with >4000 antibodies.[10] Recently, a new proteomics platform, slow off-rate modified aptamers (SOMAmers) scan, was developed for the examination of global protein expression.[11,12] SOMAmers rely on the natural 3-dimensional folding of single-stranded DNA-based protein affinity reagents. SOMAmers are deoxyoligonucleotides with unique intramolecular motifs that bind to the respective protein targets in native conformations.[13] The technology enables a simultaneous quantitative analysis of 1305 proteins per sample using small sample volumes. SOMAscan has been applied to studies on several types of medical conditions to identify proteins and molecular mechanisms involved in disease progression.[14–19] To better understand the pathogenesis associated with high mortality in AKI-D patients, we performed SOMAscan proteomic profiles on serum samples collected from 207 participants in the Veterans Affairs/National Institutes of Health Acute Renal Failure Trial Network (ATN) study.

CONCISE METHODS

Study Design

The ATN study was a prospective, multicenter randomized clinical trial that evaluated the intensity of renal replacement therapy in critically ill patients with AKI-D; it enrolled 1124 patients from 27 Veterans Affairs and 12 academic medical centers across the United States. Outcomes included 60-day mortality, recovery of kidney function, and intensive care unit and hospital length of stay. Details of the study protocol, including inclusion and exclusion criteria, have been previously published.[20,21] Patients enrolled in the ATN study were critically ill adults (18 years or older) who had AKI that was clinically consistent with acute tubular necrosis and failure of ≥1 nonrenal organ (defined as a nonrenal sequential organ failure assessment [SOFA] score of ≥2) or sepsis. Consent for sample collection and at least 1 sample were obtained from 827 of the 1124 subjects who participated in the ATN study. A total of 819 patients provided samples on day 1 and 573 patients on day 8, with 565 patients contributing samples on both day 1 and day 8. For this *post hoc* analysis, we randomly selected 100 day 1 serum samples from patients who either died before (n = 49) or survived to (n = 51) day 8 and 107 day 8 serum samples from patients who either died before (n = 24) or survived to (n = 83) day 28. Clinical data available through the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) data repository via a cross-walk file was then linked to the de-identified samples analyzed for this study. This *post hoc* analysis was approved by the Salem Veteran's Affair Medical Center and Food and Drug Administration Institutional Review Boards.

SOMAscan Proteomic Profiling

We performed quantitative proteome profiling of AKI samples using the SOMAscan assay developed by Somalogic Inc. (Boulder, CO) as described previously.[11,22] A total of 1305 serum proteins were measured simultaneously in a single assay using 50 µl of sample per the manufacturer's sample processing procedures. In each assay, 6 calibrator samples, 2 quality controls (QCs), and 1 blank sample from the assay manufacturer were included. We detected the fluorescence signal intensities of SOMAmers using an Agilent microarray scanner (Agilent Technologies, Inc., Santa Clara, CA).

Data Processing

We used SOMAscan standard calibration and normalization procedures, including hybridization control normalization, median signal normalization, and between-run calibration, to remove systematic biases. The hybridization control normalization was achieved using the global relative fluorescence units (RFUs) of each of the 12 hybridization control sequences. For median signal normalization, a scale factor was derived for each dilution set. Between-run calibration was achieved using the 6 calibrators from common pooled samples. After normalization and calibration, we stratified the day 1 samples based on survival to day 8 and stratified day 8 samples based on survival to day 28. Welch's *t*-test was performed for logarithm RFUs to find significantly changed proteins between the deceased and surviving groups. The *t*-test *P* values were also adjusted for this multiplex assay to calculate the false discovery rate (FDR) using the Benjamini and Hochberg method.[23]

Ingenuity Pathway Analyses

We used Ingenuity Pathway Analyses (IPA) software (QIAGEN, Redwood City, CA) for gene ontology, pathway analyses, and core analysis comparison for the proteins that changed significantly at day 1 in patients who died in <8 days versus those who survived >8 days. A *P* value of <0.05 was considered significant.