Development of an Immunoassay for the Kidney-Specific Protein myo-Inositol Oxygenase, a Potential Biomarker of Acute Kidney Injury

Joseph P. Gaut,1* Dan L. Crimmins,2 Matt F. Ohlendorf,2 Christina M. Lockwood,2 Terry A. Griest,2 Nancy A. Brada,2 Masato Hoshi,3 Bryan Sato,4 Richard S. Hotchkiss,4 Sanjay Jain,1,3 and Jack H. Ladenson2

BACKGROUND: Acute kidney injury (AKI) affects 45% of critically ill patients, resulting in increased morbidity and mortality. The diagnostic standard, plasma creatinine, is nonspecific and may not increase until days after injury. There is significant need for a renal-specific AKI biomarker detectable early enough that there would be a potential window for therapeutic intervention. In this study, we sought to identify a renal-specific biomarker of AKI.

METHODS: We analyzed gene expression data from normal mouse tissues to identify kidney-specific genes, one of which was Miox. We generated monoclonal antibodies to recombinant myo-inositol oxygenase (MIOX) and developed an immunoassay to quantify MIOX in plasma. The immunoassay was tested in animals and retrospectively in patients with and without AKI.

RESULTS: Kidney tissue specificity of MIOX was supported by Western blot. Immunohistochemistry localized MIOX to the proximal renal tubule. Serum MIOX, undetectable at baseline, increased 24 h following AKI in mice. Plasma MIOX was increased in critically ill patients with AKI [mean (SD) 12.4 (4.3) ng/mL, n = 42] compared with patients without AKI [0.5 (0.3) ng/mL, n = 17] and was highest in patients with oliguric AKI [20.2 (7.5) ng/mL, n = 23]. Plasma MIOX increased 54.3 (3.8) h before the increase in creatinine.

CONCLUSIONS: MIOX is a renal-specific, proximal tubule protein that is increased in serum of animals and plasma of critically ill patients with AKI. MIOX preceded the increases in creatinine concentration by approximately 2 days in human patients. Large-scale studies are warranted to further investigate MIOX as an AKI biomarker. © 2014 American Association for Clinical Chemistry

Acute kidney injury (AKI)5 is common among hospitalized and critically ill patients, and its incidence is increasing (1–3). Approximately 45% of critically ill patients and 20% of hospitalized patients develop AKI (2, 4). This results in increased hospital stays, infectious complications, and increased mortality, at significant cost (5–8). Recent studies have linked AKI with future development of chronic kidney disease (9, 10). Multiple factors contribute to the development of AKI including sepsis, ischemia, drugs, intravenous contrast, and infection (11–16).

The current standard for identifying AKI, plasma creatinine, is nonspecific and insensitive (14, 17, 18). Plasma creatinine may not increase until days after injury or 50% of renal function is lost. Plasma creatinine is unable to accurately predict glomerular filtration rate (GFR) in the non–steady state of AKI, underestimating the renal function decline. Last, because creatinine depends on muscle mass and hepatic function, its plasma concentration may differ depending on these factors (14, 17, 18).

There is a critical unmet need for a real-time, specific, and sensitive AKI biomarker. The American Society of Nephrology, Acute Dialysis Quality Initiative, and Acute Kidney Injury Network (AKIN) have prioritized the identification and validation of AKI biomarkers (3, 19). Early detection of AKI may allow for timely intervention and perhaps decrease its significant morbidity and mortality (20). Although multiple new drugs have been developed to treat AKI, they have not

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1 Department of Pathology and Immunology, Division of Anatomic and Molecular Pathology, 2 Department of Pathology and Immunology, Division of Laboratory and Genomic Medicine, 3 Department of Medicine, Renal Division, 4 Department of Anesthesiology, Washington University School of Medicine, St. Louis, MO.

* Address correspondence to this author at: Washington University School of Medicine, 660 S. Euclid Ave, Campus Box 8118, St. Louis, MO 63110. Fax 314-362-0107; e-mail gautj@path.wustl.edu.

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5 Nonstandard abbreviations: AKI, acute kidney injury; GFR, glomerular filtration rate; AKIN, Acute Kidney Injury Network; IL, interleukin; NGAL, neutrophil gelatinase-associated lipocalin; KIM-1, kidney injury molecule 1; MIOX and Miox, myo-inositol oxygenase; cRNA, complementary RNA; GST, glutathione S-transferase; MIOX, recombinant MIOX; BLAST, basic local alignment search text; H&E, hematoxylin and eosin; FABP1, liver type fatty acid–binding protein 1.
proven effective in the clinical setting (21–27). This has been attributed, in part, to the inability to detect AKI early. Multiple studies have investigated a variety of blood and urine biomarkers for the diagnosis of AKI including interleukin (IL)-18, neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule 1 (KIM-1), among others (28–32). Although substantial progress has been made, no specific, early biomarkers of AKI have translated into clinical practice.

Renal tubular proteins are ideal biomarkers of AKI, as it is well established that tubular damage is an early event in renal ischemia. Although both proximal and distal tubules are injured, the S3 segment of the proximal tubule located in the outer medulla is particularly sensitive to decreased blood flow (7, 33). When tubular injury occurs, proximal tubular proteins may leak into the extratubular space and be excreted into urine or reabsorbed into blood.

In the current study, we identified myo-inositol oxygenase (MIOX) as a kidney-specific protein highly concentrated in the proximal tubule. We developed a sensitive immunoassay to measure plasma MIOX and investigated the utility of plasma MIOX as an AKI biomarker in an animal model of AKI and samples retrospectively obtained from critically ill human patients.

Methods

GENE ARRAY ANALYSIS

We identified kidney-specific genes according to methods described previously (34). Briefly, brain, liver, spleen, kidney, skeletal muscle, lung, pancreas, heart, and small intestine were dissected from 3 C57Bl/6 mice and snap frozen in liquid nitrogen. Total RNA was isolated, converted into biotinylated complementary RNA (cRNA), fragmented, and applied to mouse MU75A (version 1) GeneChip arrays (Affymetrix). The fluorescence intensity was scaled to 1500, and the mean difference values were calculated with Affymetrix MU75A (version 1) GeneChip arrays (Affymetrix). We estimated concentrations using absorbance at 280 nm with calculated extinction coefficients. MIOX and glutathione S-transferase (GST) sequences were obtained from the Swiss-Prot website (http://www.expasy.org). Recombinant GST-MIOX was cleaved with thrombin to obtain recombinant MIOX (rMIOX) devoid of GST. We analyzed this material by denaturing gel electrophoresis and N-terminal Edman sequencing to confirm purity and identity.

ANTI-MIOX ANTIBODIES

Rabbit polyclonal antibodies were produced at Harlan Bioproducts for Science with recombinant GST-MIOX as an immunogen. Polyclonal antibodies were purified by first removing cross-reacting anti-GST with a GST column (Pierce) according to the manufacturer’s instructions. Antibodies were then affinity purified as described previously (35). Mouse monoclonal antibodies were produced at Maine Biotechnology Services with recombinant GST-MIOX as the immunogen. Monoclonal antibodies were purified by use of a protein A-agarose column, dialyzed against phosphate-buffered saline, pH 7.2, containing 0.05% NaN3, and quantified by absorbance at 280 nm. We typed monoclonal antibodies using isotyping cassettes (Pierce). Epitope mapping was performed by use of Abimed spot peptide arrays as described previously (34).

Briefly, peptide spot arrays composed of MIOX residues 1–10, 3–12, 5–14, and so on were probed with the anti-MIOX antibodies. We analyzed the epitope sequences by basic local alignment search text (BLAST) using a nonredundant Homo sapiens database and default algorithm parameters (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

WESTERN BLOTTING

Normal human kidney homogenate and premade tissue blots were purchased from G-Biosciences. We performed Western blotting as previously described (34).

Briefly, blots were probed with 4 μg/mL polyclonal or monoclonal antibody. Where indicated, both monoclonal antibodies were used simultaneously at the above concentrations. Antispecies GAR-AP and GAM-AP (Jackson ImmunoResearch Laboratories) were used as detection antibodies at a 1:1000 dilution.

IMMUNOHISTOCHEMISTRY

Formalin-fixed paraffin-embedded human kidney tissue was obtained from the Lauren V. Ackerman Laboratory of Surgical Pathology at Barnes Hospital. Samples were obtained from the uninvolved portions of kidneys resected for renal cell carcinoma. Single sections were stained after sodium citrate antigen retrieval by use of a Ventana autostainer as described previously.
We used a final concentration of 5 \( \mu \text{g/mL} \) of the rabbit polyclonal anti-MIOX antibody.

**MIOX IMMUNOASSAY**

We developed a sandwich immunoassay for MIOX using monoclonal antibody 12H06 as capture antibody and biotinylated monoclonal antibody 01D10 as a capturing antibody. We used this immunoassay to measure MIOX from human and mouse samples. Biotinylation of antibody 01D10 was performed by use of Sulfo-NHS-LC-Biotin (Pierce) according to the manufacturer’s instructions. The capture antibody was added to the plate at a concentration of 30 \( \mu \text{g/mL} \). The capturing antibody was used at a concentration of 0.33 \( \mu \text{g/mL} \). Nonspecific binding was blocked by use of Pierce Superblock, mouse immunoglobulin G (Equitech-Bio), and 0.5 g/L Tween-20 (Sigma-Aldrich). Plasma samples, controls, and standards were diluted 1:8 in Superblock, mouse IgG, and Tween-20 before analysis. GST-MIOX was serially diluted for construction of a standard curve. We determined the concentration of GST-MIOX using amino acid analysis (AAA Services Lab). Streptavidin conjugated to ruthenium (MesoScale Discovery) was added to samples and detected by use of a MesoScale Discovery Sector 2400 electrochemiluminescent plate reader. Spike recovery was determined by adding recombinant MIOX to a final concentration of either 2 or 10 ng/mL to control human heparin plasma. Dilutional linearity was evaluated by adding recombinant MIOX to a final concentration of 5 ng/mL to 1:8, 1:16, 1:32, and 1:64 dilutions of control human heparin plasma.

**ANIMAL MODEL**

All animal studies were approved by the Animal Studies Committee of Washington University School of Medicine. We used C57Bl/6 mice (3 female and 4 male) ranging in age from 8 to 12 weeks. One week before surgery, serum was collected and frozen at \(-80^\circ\text{C}\). Animals were subjected to bilateral renal ischemia for 30 min. Briefly, animals were anesthetized with a mixture of ketamine and xylazine, their body temperature was maintained at 37 \( ^\circ\text{C} \) on a heating pad and monitored with a rectal probe throughout surgery, and ischemia was induced by bilateral clamping of renal vascular pedicles for 30 min. Two sham-operated animals (1 male and 1 female) underwent an identical procedure without vascular pedicle clamping. Twenty-four hours after surgery, the animals were killed, serum was collected, and kidneys were perfused with 4% paraformaldehyde and placed in 4% paraformaldehyde (pH 7.4). Serum was stored at \(-80^\circ\text{C} \) before immunoassay as described above for human plasma. We processed tissues for routine hematoxylin and eosin (H&E) staining according to standard procedures.

**HUMAN PATIENTS**

All human studies were approved by the internal review board for human studies at Washington University School of Medicine. Over a period of 2 years, laboratory data from adult patients in the surgical or medical intensive care units were screened for increases in plasma creatinine occurring over a 24- to 72-h time period. Laboratory data were also screened from age-matched patients in the surgical or medical intensive care units or on hospital floors for stable plasma creatinine over 72 h. Patients with chronic kidney disease or who did not have Foley catheters were not included. We obtained remnant heparin plasma samples from the Clinical Chemistry Laboratory of Barnes Hospital. We obtained plasma samples before the increase in plasma creatinine (designated time 0 h) and at the time of the plasma creatinine increase (designated time 54 h). For patients with stable plasma creatinine, 1 representative sample was retrieved from the Clinical Chemistry Laboratory. Aliquots (500 \( \mu \text{L} \)) of plasma were prepared and frozen at \(-80^\circ\text{C} \) before analysis. We reviewed patient medical records for demographic information, urine output, and diagnosis. Oliguria was defined as a urine output <0.5 mL/kg/h for at least 6 h.

**STATISTICS**

Quantitative data are presented as mean (SE) unless otherwise indicated. All statistical analyses were performed by use of GraphPad Prism software. Comparison of multiple groups of patients and animals was evaluated by use of the Kruskal–Wallis test with Dunn correction for post hoc tests. Standard curves were generated by use of a 4-parameter logistic curve fit with 1/year weighting. Significance was defined as \( P < 0.05 \).

**Results**

**IDENTIFICATION OF myo-INOSITOL OXYGENASE AS A RENAL-SPECIFIC BIOMARKER**

We sought to identify mouse genes expressed in the kidney by a factor of at least 10-fold compared with other tissues. Using this strategy, we identified myo-inositol oxygenase (Miox) as a renal-specific, abundant gene (Fig. 1A). The Miox gene encodes the protein myo-inositol oxygenase (Miox). To confirm the tissue-specific nature of the human homolog of Miox, human tissue homogenates from liver, brain, lung, kidney, spleen, testis, ovary, heart, and pancreas were probed by use of an anti-MIOX rabbit polyclonal antibody. Consistent with the mouse gene profiling data, MIOX protein was detected only in human kidney homogenate (Fig. 1B).
CHARACTERIZATION OF ANTI-MIOX ANTIBODIES

Rabbit polyclonal and mouse monoclonal anti-MIOX antibodies were generated by use of recombinant GST-tagged MIOX as antigen. To confirm the identity of recombinant GST-MIOX, the protein was first cleaved into GST and MIOX with thrombin. The cleaved proteins were separated by use of SDS-PAGE and subjected to Edman sequencing (Fig. 2A). The protein migrating between 26 and 37 kDa gave a sequence of GSPEFKVTVG corresponding to the N terminus of MIOX. The protein near 26 kDa gave a sequence of MSPILGYWKI corresponding to the N terminus of *S. japonicum* GST. Both monoclonal antibodies were isotyped and found to be IgG2b/H9260.

Western blots were used to confirm the antibodies’ ability to recognize recombinant MIOX and endogenous MIOX present in normal human kidney homogenate (Fig. 2A). The polyclonal and monoclonal antibodies recognized a protein with molecular mass between 26 and 37 kDa that comigrated with the recombinant MIOX protein (Fig. 2A). These results are consistent with the expected molecular weight of MIOX, 33 kDa.

The linear epitopes of the anti-MIOX antibodies were determined by use of spot-peptide membrane arrays containing the full-length amino acid sequence of MIOX. The 2 mouse monoclonal antibodies mapped to opposite ends of the MIOX sequence (Fig. 2B). The antibody designated 01D10 mapped to the N-terminal sequence of MIOX (Fig. 2C). The mouse monoclonal antibody designated 12H06 mapped to the C-terminal region of MIOX (Fig. 2C). Human MIOX showed 89.8% homology to mouse Miox. The peptide epitope recognized by the 12H06 mouse monoclonal antibody was 100% identical to the corresponding mouse Miox peptide sequence. The epitope recognized by the 01D10 mouse monoclonal antibody differed by 1 amino acid: the valine at amino acid position 21 is replaced by a methionine in mouse Miox. The rabbit anti-MIOX polyclonal antibody R9544 demonstrated 5 regions of intense staining, a finding consistent with the polyclonal nature of this antibody (Fig. 2C). Each monoclonal epitope sequence was validated for its uniqueness in a nonredundant *Homo sapiens* database by use of BLAST. A critical output parameter from BLAST is the expected value (E value). The E value is a measure of the chance that a random alignment from the probed database would produce the same normalized score. On the basis of the E value, there is a chance of 1 in 1,000,000 chance that the epitope sequences could be randomly found in the database (data not shown). The lowest E value of a non-MIOX protein was 2.3, compared to a MIOX E value of $1 \times 10^{-6}$, indicating the highly specific nature of the antibody epitopes. Individual peptides corresponding to the mapped epitopes for both monoclonal antibodies were produced. Preincubation by use of the N-terminal peptide epitope, but not the C-terminal peptide epitope, inhibited binding of the 01D10 mouse monoclonal anti-MIOX antibody to a MIOX spot-peptide array. Similarly, preincubation by use of the C-terminal peptide epitope, but not the N-terminal peptide epitope, inhibited binding of the 12H06 mouse monoclonal anti-MIOX antibody to a MIOX spot-peptide array (data not shown).

IMMUNOHISTOCHEMICAL ANALYSIS OF MIOX IN HUMAN KIDNEY

Human kidney tissues were obtained from uninvolved portions of partial nephrectomy samples from patients with renal cell carcinoma. Single formalin-fixed paraffin-embedded tissue sections were stained with rabbit polyclonal anti-MIOX antibody. Consistent with previous studies, the MIOX pro-
Fig. 2. MIOX antibody characterization.
(A), Western blot of 10 μg normal human kidney homogenate (kidney) with the rabbit anti-MIOX polyclonal antibody and the mouse monoclonal antibodies 12H06 and 1D10. Recombinant GST-MIOX (rMIOX, 10 μg) was cut with thrombin, run on the same gel, transferred to polyvinylidene difluoride, and protein stained. The protein-stained bands were subjected to Edman sequencing and confirmed to represent MIOX and GST as indicated. The remaining lanes were cut and stained with the rabbit polyclonal anti-MIOX antibody (pAb R9544), mouse monoclonal anti-MIOX antibody 12H06 (mAb 12H06), and mouse monoclonal antibody 1D10 (mAb 1D10). Nonspecific bands were identified by use of pAb R9544 (~64 and ~22 kDa) and the mAb 12H06 (~40 kDa). The only band that reacted with all 3 antibodies corresponded to recombinant MIOX (~33 kDa). (B), Identification of anti-MIOX antibody epitopes. Spot-peptide membrane array immunostaining for rabbit polyclonal antibody R9544, mouse monoclonal antibody 01D10 (mAb 01D10), and mouse monoclonal antibody 12H06 (mAb 12H06). Each spot comprised a 10-mer synthetic peptide: spot 1, residues 1–10; spot 2, residues 3–12; spot 3, residues 5–14 (and so on) until the entire sequence was covered. The numbers to the left of the blots correspond to the spot number at the beginning of the row. *End of the sequence. (C), Representation of anti-MIOX antibody epitopes. The amino acid sequence of MIOX is shown. The epitope map of the rabbit polyclonal anti-MIOX antibody is italicized, mouse monoclonal antibody 12H06 is bold and underlined, and the mouse monoclonal antibody 1D10 is boxed. The mAb 1D10 antibody recognizes an epitope near the N terminus, and the mAb 12H06 antibody recognizes an epitope near the C terminus. The polyclonal antibody reacts with N- and C-terminal epitopes and 2 internal epitopes.

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tein showed strong cytoplasmic immunoreactivity in cells morphologically consistent with proximal tubules (Fig. 3) (37).

MIOX IMMUNOASSAY
The mouse monoclonal antibodies were optimized for use in a sandwich immunoassay. The monoclonal antibody 12H06 was used as a capture antibody and biotinylated monoclonal antibody 01D10 was used as a capping antibody. The immunoassay performance characteristics are summarized in Supplemental Table 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol60/issue5. The limit of detection, defined as a signal-to-noise ratio of 2 relative to background, was 115 (55) pg/mL [n = 13, mean (SD)]. Human plasma samples stored at 4 °C or −80 °C for 10 days showed no significant difference in MIOX concentration [absolute mean % difference = 7.4% (4.0%), mean % difference = −0.4% (9.4%); n = 4]. The interassay CV was 15.2% (3.4%) [mean MIOX concentration 5.6 (0.7) ng/mL, n = 9]. All samples underwent 2 freeze-thaw cycles. The intraassay CV, determined by use of 19 plasma samples with a mean MIOX concentration of 21.2 ng/mL, range 1.7–48.2 ng/mL, was 7.9% (5.2%). GST-MIOX spiked into human heparinized plasma demonstrated 94% (19%) recovery of the expected signal (n = 8). The MIOX immunoassay demonstrated dilutional linearity recovery of 109% (12%) (n = 9) (see online Supplemental Fig. 1).

MOUSE AKI MODEL
Miox was not detected in the serum of normal or sham-operated mice 24 h postoperatively (Fig. 4A). In contrast, Miox was markedly increased in the serum of mice 24 h after 30 min of bilateral ischemia reperfusion injury (Fig. 4A). Histologic examination of kidneys from sham-operated animals showed no evidence of tubular necrosis (Fig. 4B). In contrast, histologic examination revealed significant tubular necrosis in the kidneys of the experimental mice 24 h after injury (Fig. 4C).

ANALYSIS OF PLASMA FROM AKI PATIENTS
Patients in an intensive care unit were screened for AKI, defined according to the AKIN criteria (3). Patients were screened for plasma creatinine increases of at least 1.5 times baseline or an absolute increase of 0.3 mg/dL (0.027 mmol/L) over 1–3 days. Once a patient was identified who had an increase in plasma creatinine, remnant heparin plasma samples were collected from the Barnes Hospital Clinical Chemistry Laboratory at the time of the plasma creatinine increase and 1–3 days before the plasma creatinine increase (time 0). Plasma creatinine increased a mean (SE) of 54 (3.8) h (n = 33; time 54) after the time 0 sample. Patient characteristics are summarized in Table 1. Oliguria was defined according to the AKIN criteria as <0.5 mL/kg/h for at least 6 h (3). A total of 42 patients fulfilled criteria for at least stage 1 AKI, defined as a relative increase in plasma creatinine of 50%, an absolute increase in plasma creatinine of 0.3 mg/dL (0.027 mmol/L), or a decrease in urine output to <0.5 mL/kg/h for at least 6 h (3). Of the 42 patients with AKI, 33 had increases in plasma creatinine of at least 1.5 times baseline. Nine patients had a urine output of <0.5 mL/kg/h for at least 6 h without a change in plasma creatinine. Seventeen hospitalized or critically ill patients who did not meet...
criteria for AKI served as controls. There was no significant difference in age between the patient groups. There was a greater proportion of male patients within the AKI group. Plasma MIOX was measured in critically ill patients with AKI at time 0 h (n = 42) and time 54 h (n = 37); the mean time plasma creatinine increased (Fig. 5). Samples were not available at the time of plasma creatinine increase for 5 patients. Plasma MIOX was significantly increased in patients with AKI at time 0 [12.4 (4.3) ng/mL] and at time 54 [10.1 (5.3) ng/mL] relative to controls [0.5 (0.3) ng/mL; P = 0.002]. Patients with oliguric AKI had significantly higher plasma MIOX values at time 0 [20.2 (7.5) ng/mL, n = 23] and time 54 [17.1 (11.0) ng/mL, n = 17] compared with controls (P < 0.05) (Fig. 6). Significantly higher plasma MIOX concentrations were observed in patients with dialysis requiring AKI at time 0

Fig. 3. (A), Formalin-fixed paraffin-embedded human kidney tissue was stained with the rabbit polyclonal anti-MIOX antibody. Intense staining of the renal cortex is seen (40×). (B), On high power, cells consistent with proximal tubules (*) showed strong staining. Adjacent distal tubules (arrow), glomeruli (G), and blood vessels showed no significant immunoreactivity (400×).

Fig. 4. Mouse AKI model.
(A), Miox was measured in mouse serum from sham-operated animals, animals at baseline, and 24 hours after AKI (n = 5). Miox was below the limit of detection (LOD) [115 (55) pg/mL] in sham-operated animals and at baseline. Serum Miox was increased at 24-h postinjury [2.8 (0.7) ng/mL, mean (SE); *P < 0.03]. (B), Representative section of renal cortex from a sham-operated mouse shows intact renal tubules (H&E, 400×). (C), Representative section of renal cortex at 24-h postinjury. Extensive tubular necrosis is evident (arrows) (H&E, 400×).
An ideal AKI biomarker should be kidney specific, rapidly detectable following injury, correlated with the degree of damage, and easily measured. A variety of approaches have been used to identify such a biomarker. In the current study, we first sought to identify kidney-specific genes. Because the proximal tubule is the major site of ischemic damage, genes specific to the proximal tubule were targeted. Using differential gene expression profiling, we identified MIOX as a renal-specific proximal tubule gene. Tissue specificity of MIOX was

**Table 1. Patient characteristics.**

<table>
<thead>
<tr>
<th>No AKI</th>
<th>AKI</th>
<th>Nonoliguric AKI</th>
<th>Oliguric AKI</th>
<th>Dialysis-requiring AKI</th>
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<tr>
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<td></td>
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<tr>
<td>n</td>
<td>17</td>
<td>42</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>Age, years</td>
<td>58 (4)</td>
<td>56 (2)</td>
<td>56 (2)</td>
<td>57 (6)</td>
</tr>
<tr>
<td>M/F</td>
<td>8:9</td>
<td>24:18</td>
<td>11/14 (79)</td>
<td>11/14 (79)</td>
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<td>Unknown</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peak creatinine, mg/dL [mmol/L]</td>
<td>0.88 (0.08) [0.078 (0.007)]</td>
<td>2.03 (0.17) [0.179 (0.015)]</td>
<td>1.55 (0.12) [0.172 (0.011)]</td>
<td>1.81 (0.19) [0.160 (0.017)]</td>
</tr>
<tr>
<td>Peak blood urea nitrogen, mg/dL [mmol/L]</td>
<td>16.2 [5.7 (0.7)]</td>
<td>36.3 [12.9 (1,1)]</td>
<td>38.4 [13.3 (1.4)]</td>
<td>31.0 [11.1 (0.7)]</td>
</tr>
</tbody>
</table>

**Fig. 5. MIOX in critically ill and hospitalized patients.**

(A), Plasma creatinine (Cr) peaked 54.3 (3.8) h (time 54) relative to the preceding Cr measurement (time 0) and was increased at time 54 in patients with AKI (**P < 0.005).**

(B), Patients with AKI showed higher plasma MIOX concentrations at time 0 and time 54 compared with patients without AKI (*P < 0.002). To convert creatinine in mg/dL to mmol/L, multiply by 0.0884.

[8.2 (3.5) ng/mL, n = 5] and time 54 [8.2 (3.1) ng/mL, n = 5] compared with controls (P < 0.05) (Fig. 6). All plasma MIOX concentrations were determined before initiation of dialysis. The increase in plasma MIOX preceded the increase in plasma creatinine by a mean of 54.3 (3.8) h (n = 33).

**Discussion**

An ideal AKI biomarker should be kidney specific, rapidly detectable following injury, correlated with the degree of damage, and easily measured. A variety of approaches have been used to identify such a biomarker. In the current study, we first sought to identify kidney-specific genes. Because the proximal tubule is the major site of ischemic damage, genes specific to the proximal tubule were targeted. Using differential gene expression profiling, we identified MIOX as a renal-specific proximal tubule gene. Tissue specificity of MIOX was
confirmed by use of Western blot. MIOX localized to the renal proximal tubule by use of immunohistochemistry. Mouse monoclonal anti-MIOX antibodies were developed and characterized. These monoclonal antibodies were optimized for use in a sandwich immunoassay to quantify MIOX in plasma. This assay was used to investigate MIOX as a biomarker of AKI. Mice subjected to bilateral renal ischemia-reperfusion showed increased plasma MIOX at 24-h postinjury. Critically ill patients with AKI showed increased plasma MIOX compared with control patients without AKI. Serum MIOX was highest in patients with oliguric and dialysis-requiring AKI. The increase in plasma MIOX occurred approximately 2 days before the increase in plasma creatinine.

MIOX is a renal-specific enzyme that catalyzes the first committed step in myo-inositol metabolism (38). The MIOX mRNA transcript is reportedly downregulated in a rat model of ischemic AKI (37). In this study, the authors hypothesized that MIOX mRNA loss was directly related to the degree of necrosis following renal ischemia. In the current study, demonstration of increased plasma MIOX following AKI may also be related to necrosis of the proximal tubule, although secretion cannot be ruled out.

It is noteworthy that MIOX was not detected in plasma of all patients with AKI defined by use of the AKIN criteria (3). Because the timing of renal injury was unknown in this patient population, it is possible that an increase in plasma MIOX may have occurred before or after samples were obtained. Plasma creatinine is a nonspecific biomarker of renal injury, so it is possible that a subset of patients developed increased plasma creatinine in the absence of proximal tubule cell damage.

Other proteins have been investigated as potential kidney injury biomarkers. Previous studies demonstrated that NGAL, KIM-1, α-GST, liver type fatty acid–binding protein 1 (FABP1), and N-acetyl-β-D-glucosaminidase are detectable in urine following renal injury (32, 39, 40). However, none of these has yet translated into clinical use. In contrast to these bio-

**Fig. 6.** (A), Plasma creatinine is similar among all groups at time 0. (B), Plasma MIOX increased at time 0 in patients with oliguric and dialysis-requiring AKI. (C), Plasma creatinine increased at time 54 in patients with AKI. (D), Plasma MIOX was increased at time 54 in patients with oliguric and dialysis-requiring AKI relative to patients without AKI. *P < 0.05. To convert creatinine in mg/dL to mmol/L, multiply by 0.0884.
markers, MIOX is a kidney-specific protein. MIOX is also an endogenous kidney protein. Therefore, it may be released more rapidly than the inducible biomarkers KIM–1, NGAL, and FABP1. Measuring these analytes together may provide more detailed information regarding the timing of renal injury. Interestingly, MIOX did not appear to signal nonoliguric kidney injury in this retrospective study. It will be important to confirm this result in a prospective study. Nonetheless, it is most likely that a panel of biomarkers will be necessary to accurately detect AKI, determine severity, and localize the portion of the nephron injured in a given clinical scenario.

There are several limitations to this study. The human samples were collected in a retrospective manner from critically ill and hospitalized patients. The study population was screened for patients with clinical evidence of AKI and is therefore not an accurate representation of all critically ill patients. Because these samples were obtained retrospectively, they were not immediately frozen after collection. Rather, the samples were stored at 4 °C for 1–7 days before aliquoting and freezing. Although the MIOX immunoassay does not appear to be significantly affected by storing plasma for up to 10 days at 4 °C, it is important to note that the samples were not all handled identically. This may have introduced artifact into the analysis. Previous studies confirmed the kidney-specific nature of MIOX. However, it is unknown whether MIOX mRNA concentration patterns may change in other tissues following AKI or multiorgan failure. Additional studies are necessary to investigate the tissue profile of MIOX in these settings to explore the renal-specific nature of its origin.

In conclusion, we developed an immunoassay to quantify the kidney-specific protein MIOX in human plasma. We demonstrated that MIOX was increased in the serum of animals and the plasma of critically ill patients with AKI. In critically ill patients, MIOX increased approximately 2 days before the increase in plasma creatinine, potentially opening a therapeutic window. Additional studies are warranted to further investigate the potential of MIOX as an early, kidney-specific biomarker of AKI.

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