Tubular Expression of KIM-1 Does not Predict Delayed Function After Transplantation


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ABSTRACT

Injured epithelial cells of the proximal tubule upregulate the glycoprotein kidney injury molecule 1 (KIM-1), suggesting its potential as a biomarker of incipient kidney allograft injury. It is unknown whether KIM-1 expression changes in kidney allografts with delayed graft function (DGF), which often follows ischemia-reperfusion injury. Here, we prospectively measured KIM-1 RNA and protein expression in preperfusion biopsies of 30 living- and 85 deceased-donor kidneys and correlated the results with histologic and clinical outcomes after transplantation. We detected KIM-1 expression in 62% of deceased-donor kidneys and only 13% of living-donor kidneys (P < 0.0001). The level of KIM-1 expression before reperfusion correlated inversely with renal function at the time of procurement and correlated directly with the degree of interstitial fibrosis. Surprising, however, we did not detect a significant correlation between KIM-1 staining intensity and the occurrence of DGF. Our findings are consistent with a role for KIM-1 as an early indicator of tubular injury but do not support tissue KIM-1 measurement before transplantation to identify kidneys at risk for DGF.


Delayed graft function (DGF) is a frequent complication of transplantation and is most commonly caused by acute tubular necrosis. The incidence of DGF varies across studies and is, to a large extent, definition dependent. For example, when DGF is defined by the need for dialysis within the first week after transplantation, United Network for Organ Sharing data indicate that the incidence is approximately 23%. Irrespective of the definition, the occurrence of DGF is associated with an increased risk for graft loss, acute rejection, and chronic allograft nephropathy. The rate of DGF may intensify as a result of increasing interest in the use of donation after cardiac death (DCD) and expanded-criteria donor kidneys (ECD); therefore, an accurate predictor of donor kidney risk for subsequent DGF may be advantageous. Clinical nomograms that may assist in re-

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cipient risk stratification exist, and although are of limited utility could be improved by the addition of biomarkers.9

The identification of a biomarker that predicts early graft dysfunction has the potential to affect organ allocation and recipient management and guide development of therapeutic interventions.10 Recent studies identified potential biomarker candidates for the diagnosis of acute kidney injury11,12; however, none of these markers has been rigorously assessed in preimplantation kidney biopsies.

One such molecule is kidney injury molecule 1 (KIM-1), which encodes a type 1 cell membrane glycoprotein that contains both an Ig-like and a mucin-like domain. Its expression is markedly upregulated in the proximal tubule in the postischemic rat kidney and is also a sensitive biomarker of acute tubular injury in native and transplanted kidneys in humans.12–15 KIM-1 protein expression was quantified in a cohort of 67 kidney allograft biopsies performed as protocol or for-cause biopsies.15 KIM-1 expression was present in all biopsies with fibrosis; however, there was no significant correlation between the occurrence of DGF and KIM-1 staining intensity.

RESULTS

Deceased Donor and Allograft Recipient Characteristics According to Immediate Graft Function

Table 1 compares characteristics of patients with immediate graft function and DGF. The overall incidence of DGF as defined by the need for dialysis within the first week was 36.5%. As expected, patients with DGF received a kidney with a higher donor creatinine level at the time of procurement. There were no differences in the proportion of ECD and DCD kidneys or cold ischemia time (CIT) between these two groups.

Expression of KIM-1 in Preperfusion Biopsies

Using immunohistochemistry, KIM-1 protein expression was undetectable in 87% of living-donor kidneys, and 13% had a more sensitive than conventional histologic analysis of protocol biopsies after transplantation for detecting early tubular injury.15 In this study, we used molecular and immunohistochemical techniques to characterize the expression of KIM-1 in preperfusion transplant biopsies and to correlate KIM-1 expression with histologic parameters. We herein provide the first evidence that preperfusion-expressed KIM-1 is linked with kidney function at the time of procurement and the degree of interstitial fibrosis; however, there was no significant correlation between the occurrence of DGF and KIM-1 staining intensity.

<table>
<thead>
<tr>
<th>Table 1. Deceased-donor and allograft recipient characteristics according to immediate graft function</th>
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<tr>
<td><strong>Characteristic</strong></td>
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<tr>
<td><strong>Donor parameters</strong></td>
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<tr>
<td>age (yr; mean SD)</td>
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<tr>
<td>age &gt;50 yr (n [%])</td>
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<tr>
<td>cause of death (n [%])</td>
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<tr>
<td>CVA</td>
</tr>
<tr>
<td>trauma</td>
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<tr>
<td>other</td>
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<tr>
<td>duration of brain death (h; mean ± SD)</td>
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<tr>
<td>final serum creatinine (mg/dl; mean ± SD)</td>
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<tr>
<td>final eGFR (ml/min; mean ± SD)</td>
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<tr>
<td>female gender (n [%])</td>
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<tr>
<td>white (n [%])</td>
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<tr>
<td>WIT (min; mean ± SD)</td>
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<tr>
<td>CIT (h; mean ± SD)</td>
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<tr>
<td>DCD (n [%])</td>
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<tr>
<td>ECD (n [%])</td>
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<tr>
<td>fibrosis (n [%]; mean ± SD)</td>
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<tr>
<td><strong>Recipient parameters</strong></td>
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<tr>
<td>age (yr; mean ± SD)</td>
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<tr>
<td>female gender (n [%])</td>
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<tr>
<td>white (n [%])</td>
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<tr>
<td>current PRA (n [%]; mean ± SD)</td>
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<tr>
<td>HLA-MM (mean ± SD)</td>
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<tr>
<td>induction therapy (n [%])</td>
</tr>
<tr>
<td>eGFR 12 mo (ml/min; mean ± SD)</td>
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<tr>
<td>graft survival 12 mo (n [%])</td>
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</table>

*Defined as dialysis within the first week. CVA, cerebrovascular accident; IGF, immediate graft function; PRA, panel-reactive antibody; WIT, warm ischemia time.
low score of 0.5 \((n = 30)\). In contrast, KIM-1 expression was detectable in 62% of deceased-donor grafts. Because KIM-1 is a transmembrane protein, it is expressed along the luminal surface membranes of injured proximal tubules (Figure 1). Among KIM-1–expressing kidneys \((n = 53)\), 40% had a score of 0.5, 32% had a score of 1, 24% had a score of 2, and 4% had a score of 3.

**Correlation of KIM-1 with Clinical and Histologic Parameters**

**Living-Donor Kidneys.**

The average age of the living donors was 40.2 ± 2.2 yr (range 18 to 58 yr). In our living-donor cohort, the mean warm ischemia time was 27.6 ± 8.6 min, and the maximum duration was 44 min. The mean CIT was 37.0 ± 5.1 min, and the maximum duration was 157 min. Four of 30 live-donor kidneys had a focal and weak KIM-1 staining (score of 0.5). We analyzed donor age, GFR, and warm ischemia time/CIT and found no correlation with the KIM-1 positivity.

**Deceased-Donor Kidneys.**

Correlations between deceased-donor tubular KIM-1 expression with clinical and histologic parameters are shown in Table 2. KIM-1 protein expression was highly positively associated with its mRNA expression, confirming our scoring system and assessment of global KIM-1 expression \((r^2 = 0.16, P = 0.001)\). With respect to clinical parameters, donor kidney function, measured by the final donor creatinine level, did not correlate with KIM-1 score; however, consistent with previous data in posttransplantation biopsies,15 the final donor creatinine clearance, a more precise marker for kidney function, was inversely and significantly correlated with KIM-1 score (Table 2).

Interstitial fibrosis is usually associated with tubular atrophy, and atrophic tubules often express KIM-1; therefore, only KIM-1 expression in intact tubules was counted for scoring. Interestingly, KIM-1 expression had a significant positive correlation with the degree of interstitial fibrosis (Table 2).

The average duration of brain death (excluding DCD) was 27.5 ± 18.3 h and ranged from 2.3 to 131.1 h. The brain death duration did not correlate with KIM-1 score \((r^2 = 0.014, P = 0.32; \text{Table 2})\). Moreover, there was no correlation with the duration of brain death and the occurrence of DGF \((r^2 = 0.00048, P = 0.85)\). Of note, the five DCD kidneys had KIM-1 scores of 0 × 2, 0.5 × 1, and 1 × 2.

The CIT ranged from 2.6 to 36.4 h (mean 17.9 ± 7.9 h) in our cohort. No correlation was found between the KIM-1 expression and CIT, suggesting the failure of protein upregulation under cold and hypoxic conditions.

In addition to the subjective dialysis-based definition of DGF and because even slow recovery of renal function after transplantation has been associated with poor late outcomes, we correlated KIM-1 expression with the creatinine reduction ratio,18 The creatinine reduction ratio at 24 and 48 h after transplantation did not correlate with the KIM-1 score \((24 \text{ h: } r^2 = 0.11, P = 0.86; 48 \text{ h: } r^2 = 0.003, P = 0.66)\). In addition, the 3-, 6-, and 12-mo estimated GFR (eGFR) did not correlate with the KIM-1 score.

In a subgroup of 38 patients, we analyzed urine samples at day 1 and/or day 2 after transplantation for cleaved KIM-1 ectodomain levels (uKIM-1). The uKIM-1 was expressed as an absolute value. Previous data showed that similar results were obtained when uKIM-1 was normalized to urinary creatinine or expressed as an absolute value.19 There was no significant difference in the average uKIM-1 excretion for living donors \((1110 \text{ ng/d; } n = 18)\) versus deceased donors \((1323 \text{ ng/d; } n = 20; P = 0.8)\). In addition, there was no correlation between uKIM-1 and tubular KIM-1 score in living donors \((r^2 = 0.0004, P = 0.81; \text{data not shown})\) or in

**Figure 1.** Representative kidney sections from four different donors are based on the KIM-1 staining of proximal tubules (inserts). (A) Score 0.5 defined as focal KIM-1 staining covering a portion of proximal tubules. (B) Score 1, weak but entire luminal staining. (C) Score 2, moderate staining. (D) Score 3, strong staining. Glomeruli and distal nephron tubules stained negatively for KIM-1 in all cases.
deceased donors \((r^2 = 0.0064, P = 0.75; \text{Table 2})\). Using a 2 × 2 contingency table, we found no systematic association between the KIM-1 positivity and the presence or absence of DGF (Table 3).

We also analyzed other donor factors, such as the use of vasopressors or cardiopulmonary resuscitation (CPR). Vasopressors were used in 82% and CPR in 24% of the donors before organ procurement. There was no correlation between KIM-1 expression and either of these parameters \((P = 0.12\) and \(P = 1.0\), respectively). For these analyses, we defined KIM-1 positivity in deceased donors as a score of \(>0.5\) because all living donors had a KIM-1 score of \(\leq 0.5\). In addition, there was no association between acute rejection rate and KIM-1 positivity (data not shown).

**Correlation of Fibrosis with Clinical Outcome**

To assess whether differences in graft outcome were related to disparities in baseline histology,\(^2\) we performed a histologic analysis on all preanastomosis deceased-donor kidney biopsies. Consistent with previous data, the degree of fibrosis was not different between kidneys that did or did not develop DGF.\(^3\) Late posttransplantation function, assessed by the 3-, 6-, and 12-mo eGFR, did not correlate with the degree of fibrosis (3 mo: \(r^2 = 0.0624, P = 0.54\); 6 mo: \(r^2 = 0.0016, P = 0.77\); 12 mo: \(r^2 = 0.0006, P = 0.84\); Table 2).

Because the KIM-1 score positively correlated with fibrosis and the final donor eGFR, we analyzed the correlation between eGFR and fibrosis. The final donor eGFR was weakly correlated with the KIM-1 score \((r^2 = 0.038, P = 0.08)\).

**DISCUSSION**

This is the first prospective, observational, cohort study to test the relationship of preimplantation tissue KIM-1 expression with immediate and long-term graft function in patients after kidney transplantation. We specifically assessed the utility of this kidney-specific injury marker before reperfusion, because such a biomarker may have implications for organ allocation and recipient management.

In this study, tubular KIM-1 expression correlated with eGFR at the time of kidney procurement but did not correlate with the incidence of DGF. There are several potential reasons for the failure of KIM-1 expression to predict DGF in this study. First, superficial wedge biopsy samples were used for analysis. Such biopsies are procured from a random spot on the kidney and in contrast to needle biopsies do not usually sample either the full thickness of the cortex or the medulla.\(^2\) In addition to the known marked sampling variability of any biopsy specimen, these biopsies do not allow analysis from the outer medulla, where there may be considerable injury.\(^2\) Second, the relative contribution of proximal versus distal tubule in human acute kidney injury is unclear, and it is important to note that KIM-1 is expressed only in the injured proximal epithelial cells.\(^2\) Third, in addition to tubular cells, endothelial cells are known to contribute to ischemia-reperfusion injury. There is experimental evidence that endothelin mediates vasospasm that might perpetuate posttransplantation renal injury.\(^2\) Of note, patients with DGF were found to have high serum

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**Table 2.** Correlations between deceased-donor tubular KIM-1 expression with clinical and histologic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preimplantation Tubular KIM-1 Score ((n = 85))</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(r^2 (r))</td>
</tr>
<tr>
<td>Tubular KIM-1 mRNA</td>
<td>0.16 (0.400)</td>
</tr>
<tr>
<td>uKIM-1*</td>
<td>0.0064 (−0.080)</td>
</tr>
<tr>
<td>Duration of brain death (h)</td>
<td>0.014 (−0.117)</td>
</tr>
<tr>
<td>Final donor serum creatinine (mg/dl)</td>
<td>0.014 (0.120)</td>
</tr>
<tr>
<td>Final donor eGFR (ml/min)</td>
<td>0.059 (−0.244)</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>0.08 (0.280)</td>
</tr>
<tr>
<td>WIT (min)</td>
<td>0.017 (0.130)</td>
</tr>
<tr>
<td>CIT (hours)</td>
<td>0.017 (−0.130)</td>
</tr>
<tr>
<td>Creatinine reduction (%)h</td>
<td>0.11 (0.010)</td>
</tr>
<tr>
<td>24 h</td>
<td>0.003 (0.054)</td>
</tr>
<tr>
<td>48 h</td>
<td>0.000 (0.007)</td>
</tr>
<tr>
<td>eGFR (mo)</td>
<td>0.016 (−0.130)</td>
</tr>
</tbody>
</table>

*Defined as dialysis.

| Table 3. Deceased-donor tubular KIM-1 and DGF rate |
|----------|-------------------|
| KIM-1 Score | IGF (n = 54) | DGF* (n = 31) | \(P\) |
| Negative | 35 (65%) | 18 (58%) | 0.062 |
| Positiveb | 19 (35%) | 13 (42%) | 0.64 |

*Defined as KIM-1 score of \(>0.5\).

bDefined as KIM-1 score of \(>0.5\).

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DISCUSSION

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In this study, tubular KIM-1 expression correlated with eGFR at the time of kidney procurement but did not correlate with the incidence of DGF. There are several potential reasons for the failure of KIM-1 expression to predict DGF in this study. First, superficial wedge biopsy samples were used for analysis. Such biopsies are procured from a random spot on the kidney and in contrast to needle biopsies do not usually sample either the full thickness of the cortex or the medulla. In addition to the known marked sampling variability of any biopsy specimen, these biopsies do not allow analysis from the outer medulla, where there may be considerable injury. Second, the relative contribution of proximal versus distal tubule in human acute kidney injury is unclear, and it is important to note that KIM-1 is expressed only in the injured proximal epithelial cells. Third, in addition to tubular cells, endothelial cells are known to contribute to ischemia-reperfusion injury. There is experimental evidence that endothelin mediates vasospasm that might perpetuate posttransplantation renal injury. Of note, patients with DGF were found to have high serum...
levels of endothelin-1. Finally, evaluating biomarkers before reperfusion fails to include alloantigen-dependent and -independent injury that occurs after reperfusion.

It is important to note that our study was designed to predict, not detect, kidney dysfunction. The current paradigm indicates that DGF after kidney transplantation not only is an early marker of organ quality, preservation, and ischemia but also represents a combined response to a series of reperfusion, inflammatory, and immunologic injuries after transplantation. Inflammation, in particular, is considered to be an important component of the acute kidney injury process that may initiate or exacerbate an alloimmune response. Our data suggest that assessing KIM-1 before reperfusion fails to include factors that affect kidney injury that occurs after reperfusion. Our observation that the preanastomosis tissue KIM-1 score did not correlate with urinary KIM-1 excretion during the first 2 d after transplantation supports this hypothesis. Wedge biopsies might underestimate total kidney KIM-1 expression. Each proximal tubule segment has a different sensitivity to injury. Pars recta including S2 segment in medullary rays and S3 segment (not included donor biopsies) in outer stripe of outer medulla are more vulnerable than pars convoluta (S1 segment and initial portion of S2 segment) and thus show more KIM-1 expression (unpublished observations, P.L.Z.). It is also important to note that KIM-1 generation and shedding into the urine are active, energy-requiring processes. In the immediate phase after kidney transplantation, uKIM-1 might not be cleaved in proportion to its synthesis.

We found no significant difference in deceased- and living-donor kidney uKIM-1 excretion. It is important to note that urinary biomarker concentrations can be influenced by the graft as well as the native kidneys and, more important, can vary depending on urinary flow.

Very recently, a study analyzed kidney KIM-1 gene expression and urinary KIM-1 in 20 brain death kidney donors before organ removal and compared these with living donors before nephrectomy. Time points analyzed were at the time of brain death diagnosis as well as just before organ retrieval. There were no differences at the time of brain death, but tissue KIM-1 mRNA was 2.5-fold and urinary KIM-1 was two-fold higher in brain death donors when compared with living donors. This study was too small to analyze DGF but was able to link urinary KIM-1 as a positive predictor of 14-wk and 1-yr posttransplantation serum creatinine. We extended this study by analyzing the effects of hemodynamic instabilities and hormone dysregulation caused by brain death. In our study, KIM-1 score was not significantly associated with the duration of brain death and the need of vasopressors or CPR.

In addition, we found that the KIM-1 score assessed at the end of the CIT did not correlate with the duration of CIT. This is consistent with experimental data that energy is required for the generation of KIM-1, a commodity in short supply in the cold-preserved deceased-donor kidney.

The histologic evaluation of kidney tissue remains central to the determination of organ quality, particularly when considering ECD kidneys. Here, we used a sophisticated computerized morphometric technique for assessing global fibrosis of the biopsies. We found that renal fibrosis scores were weakly associated with the final creatinine clearance, supporting its implementation in some donor scoring systems. Interestingly, we found that fibrosis positively correlated with KIM-1 scores, suggesting increased susceptibility of kidneys with fibrosis to ischemic injury. Our findings are in accordance with previous animal studies demonstrating that focal KIM-1 expression correlated with interstitial fibrosis and proliferation; therefore, KIM-1 may play an important role in the restoration of the morphologic integrity and function of the kidney.

It is known that the ectodomain of KIM-1 is shed from cells, and this soluble form of KIM-1 has been shown to be a useful biomarker for early diagnosis of the tubular injury and predict adverse clinical outcomes in patients with acute kidney injury. It should also be noted that urinary excretion of KIM-1 has been shown to be an independent predictor of long-term kidney allograft loss in a longitudinal study of 145 transplant recipients over 4 yr. Our sample size of available urinary KIM-1 data did not have the power to detect any difference of this marker with clinical outcome, but these studies are under way.

In summary, this study showed that KIM-1 expression scored on a preperfusion wedge biopsy sample was a marker for kidney injury at the time of organ procurement, however, was not predictive for DGF.

CONCISE METHODS

Patients

This investigation was approved by the institutional review boards of the participating centers. Patients who underwent living- and deceased-donor kidney transplants between 2005 and 2007 were prospectively enrolled after written informed consent was obtained. The clinical data collected included recipient and donor age, gender, race, original kidney disease, time on dialysis before transplantation, panel-reactive antibodies, HLA matching, donor source, warm ischemia time, CIT, panel-reactive antibody status, and acute rejection. Potential living donors with hypertension are routinely excluded in our centers, and measured GFR has to be >80 ml/min. For deceased donors, data pertaining to the duration of brain death, CPR, and need for vasoconstrictors was also collected. eGFR was calculated by the abbreviated formula provided by the Modification of Diet in Renal Disease study. The immunosuppressive regimen consisted of induction therapy (68% rabbit ATG or IL-2r), intravenous methylprednisolone, and a calcineurin inhibitor with mycophenolate with or without maintenance prednisone. The presence of DGF was defined as the need for at least one dialysis treatment within the first 7 d...
after transplantation. Patients with early C4d+ acute humoral rejection and recipients of more than one previous kidney transplant were excluded from the analysis.

Processing of Kidney Biopsy Samples

Wedge biopsies were obtained at the end of the CIT. Tissue for molecular analysis was placed in RNAlater (Ambion, Austin, TX) stabilization solution and stored at −20°C. A tissue specimen was placed in 4% paraformaldehyde for histologic analysis.

Quantitative Reverse Transcriptase–PCR

Kidney tissue was removed from RNAlater and homogenized, and the RNA was extracted on a column using the RNeasy-4PCR Kit (Ambion). After DNase treatment (Ambion), the RNA was measured by spectrophotometric analysis. RNA input of 50 ng was used to synthesize cDNA with the use of iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA). No-reverse transcriptase controls were included for each sample to verify the absence of genomic DNA. A no-template control was also included in each cDNA synthesis event. Quantitative reverse transcriptase–PCR was performed using SYBR Green (Bio-Rad) on the iCycler iQ system (Bio-Rad). Each sample was tested in triplicate. All primers were designed with Beacon Designer 5 (PREMIER Biosoft Int., Palo Alto, CA) and manufactured by Integrated DNA Technologies (Coralville, IA); their sequences are available upon request. By using normal human kidney cDNA (Spring Bioscience, Fremont, CA), each primer set was evaluated to determine the appropriate annealing temperature. Dilution curves were tested in triplicate to establish the percentage efficiency. Glyceraldehyde-3-phosphate dehydrogenase served as the reference gene. β-Actin was also evaluated as a reference gene, and the results were comparable to the results with glyceraldehyde-3-phosphate dehydrogenase as reference gene (data not shown).

Histologic and Immunohistochemical Staining for KIM-1

The renal biopsies were fixed in formalin and paraffin embedded. The tissue blocks were cut into 3-μm sections and underwent routine staining for hematoxylin-eosin staining (three sections), periodic acid-Schiff (PAS) staining (three sections), and Masson Trichrome staining (one section). For each block, one 3-μm section was dewaxed in xylene and rehydrated with graded ethanol to water. AKG7, a mouse mAb against the human KIM-1 ectodomain, was used. Global KIM-1 expression was assessed by choosing the area of highest staining density and was graded by an investigator (P.L.Z.) who was blinded to the group assessments as follows: 0 to 3+ (0, no staining; 0.5, focal weak fine granular staining; 1, weak fine granular staining; 2, moderate granular staining; and 3, strong large granular staining; as in positive control) as described previously. For measurements, 30 μl of urine samples was analyzed in duplicate. The lowest limit of detection for this assay was 44 pg/ml. The inter- and intra-assay variability was <10%. The urinary KIM-1 level was expressed in absolute terms (ng/d).

Statistical Analysis

Results are expressed as means ± SD, unless stated otherwise. Comparisons of continuous variables between groups were performed by nonparametric tests and of categorical variables by two-sided χ2 or two-sided Fisher exact test where applicable. Correlation analyses between KIM-1 scores and clinical outcomes or mRNA expression were performed using the bivariate correlation method calculating the Pearson correlation coefficient. P < 0.05 was considered as statistically significant. Statistical analysis was performed with the SPSS 16.0 software package (SPSS, Chicago, IL).

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DISCLOSURES

J.V.B. is a coinventor on KIM-1 patents that have been licensed by Partners Health Care to Johnson and Johnson, Genzyme, and Biogen Idec.

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