Urinary [TIMP-2]·[IGFBP7] – Novel Biomarkers to Predict Acute Kidney Injury

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Key Words

Acute tubular necrosis · Biomarker · Emergency department · Urine microscopy · [TIMP-2]·[IGFBP7] · Urine sediment

Abstract

Background: Urine microscopy is an established technique to assess kidney disease, and can add valuable information about the mechanism of damage. However, it requires the time and expertise of an experienced nephrologist and, therefore, is typically used for a limited number of patients in practice. A rapid biomarker test that identifies patients from the emergency department (ED) who are likely to have positive urine microscopy findings would enable more efficient use of this technique. **Methods:** Four hundred patients were enrolled in the ED; thereof 362 patients had available both tissue inhibitor of metalloproteinases-2 and insulin-like growth factor-binding protein 7 [TIMP-2]·[IGFBP7] and urine score (U-Score) data at enrollment. U-Score was assessed through urine microscopy as described previously. **Results:** Fifteen (4%) of 362 patients had a U-Score >0. When patients were stratified into 3 groups using the validated [TIMP-2]·[IGFBP7] cutoffs of 0.3 and 2.0, the proportion of patients with a positive U-Score increased across the 3 strata from 1 to 6 to 24% ($p < 0.001$). At the 0.3 cutoff, [TIMP-2]·[IGFBP7] had a sensitivity of 87%, specificity of 62% and negative predictive value (NPV) of 99% for prediction of a positive U-

Score. At the 2.0 cutoff, specificity increased to 95% and positive predictive value (PPV) increased to 24%. **Conclusions:** In ED patients, urinary [TIMP-2]·[IGFBP7] had a high NPV (99%) for ruling out a positive U-Score using the 0.3 cutoff and had a PPV of 24% (6-fold greater than the pre-test probability) using the 2.0 cutoff. As such, urinary [TIMP-2]·[IGFBP7] may enable more effective use of urine microscopy in these patients and thereby save time and personnel resources.

Summary: Urine microscopy is an established technique to assess acute kidney injury and can add valuable information about the mechanism of damage; however it requires the time and expertise of an experienced nephrologist and, therefore, is typically used for a limited number of patients in clinical practice. We have shown in ED patients, urinary [TIMP-2]·[IGFBP7] had a high NPV (99%) for ruling out a positive U-Score using the 0.3 cutoff and had a PPV of 24% (6-fold greater than the pre-test probability) using the 2.0 cutoff. As such, urinary [TIMP-2]·[IGFBP7] may enable more effective use of urine microscopy in these patients and thereby save time and personnel resources.

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Introduction

In-hospital acute kidney injury (AKI) remains a common, life-threatening and expensive manifestation [1, 2]. Microscopic examination of urine sediment is a well-es-

tablished and inexpensive technique for differential diagnosis of AKI [3]. A quantitative score based on urine sediment analysis has been shown to be a strong predictor of acute tubular necrosis (ATN), the most common cause for AKI in critical ill patients, and to correlate with the severity of AKI [4]. However, the application of this technique has become less common, may be because it requires the time and expertise of an experienced examiner [5, 6].

In the last several years, new potential biomarkers for early detection of AKI have been identified [7]. Most promising are 2 markers of G1 cell cycle arrest, tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7). Multiple studies have shown that the product of the concentration of these proteins [TIMP-2]·[IGFBP7] in urine is an early indicator of risk for AKI [8–12]. Additionally, urinary [TIMP-2]·[IGFBP7] was recently cleared by the US Food and Drug Administration (FDA) as the first available AKI-biomarker test in the US [13].

Although urine microscopy is not sensitive for early detection of AKI, it informs on the nature and extent of kidney injury [14]. As such, the use of urine microscopy with protein biomarkers of AKI risk may improve AKI diagnosis. Few studies have combined urine sediment microscopy with novel protein biomarkers [14–16], and until now, no studies have investigated the combined use of urine sediment and urinary [TIMP-2]·[IGFBP7]. Others have suggested that this combination may improve risk prediction of AKI [3, 17].

We previously reported on the performance of [TIMP-2]·[IGFBP7] for risk assessment of AKI in patients presenting to the emergency department (ED) [12]. In this secondary analysis of the ED cohort, we examine the ability of urinary [TIMP-2]·[IGFBP7] to identify patients with a positive urine sediment score (U-Score). A rapid biomarker test, such as [TIMP-2]·[IGFBP7], that indicated likelihood of positive urine microscopy findings would enable more efficient use of urine sediment analysis, which can provide insight into AKI type and severity.

Subjects and Methods

We screened 400 patients presenting to the ED of the Robert Bosch Hospital (Stuttgart, Germany) as described elsewhere [12]. Admitted patients at least 18 years of age, with hemoglobin ≥ 9.5 g/dl (women) or 10.5 g/dl (men), who were capable and willing to sign an informed consent were included. Patients who required dialysis, were pregnant or failed to meet any of the inclusion criteria were excluded. This study was authorized by the Ethics Committee of the University of Tübingen. All patients were enrolled under written informed consent.

Table 1. U-Score distribution

U-Score	n	%
0	347	96
1	10	3
2	4	1
3	1	<1
4	0	0
Total	362	

Sample Collection

Fresh urine for sediment analysis was collected at the earliest possible time, centrifuged and examined by an experienced investigator under the supervision of a nephrologist immediately after receipt from the patient. Urine samples for biomarker testing were collected at enrollment and 6 and 24 h after. These samples were frozen (within 0.5 h), stored at $\leq -70^{\circ}\text{C}$ and shipped on dry ice to Astute Medical (San Diego, Calif., USA) for testing by technicians blinded to clinical data. The earliest available sample was used for the analyses in this study, which was the enrollment sample for 94%, 6-hour sample for 4% and 24-hour sample for 2% of the patients.

U-Score Calculation

For quantification, we used an established U-Score. The scoring system was applied as described previously [4]. The score is calculated by counting the renal tubular epithelial (RTE) cells (per high power field) and the granular casts (per low power field). Points are assigned from 0 to 2. The sum of both points results in the U-Score (table 1).

Biomarker Assays

Urinary TIMP-2 and IGFBP7 concentrations were measured using the NephroCheck™ Test and Astute140 Meter (Astute Medical, San Diego Calif., USA). [TIMP-2]·[IGFBP7] levels are reported in units of $(\text{ng/ml})^2/1,000$. Serum creatinine was measured at the Robert Bosch Hospital.

Statistical Analysis

The primary objective was to assess the ability of [TIMP-2]·[IGFBP7] to predict a positive U-Score. Exact Cochran-Armitage Trend Test was used to examine the association between the levels of [TIMP-2]·[IGFBP7] and U-Scores. Predictive performance was assessed using receiver operating characteristic (ROC) curve analysis, including determination of the operating characteristics at the previously validated 0.3 and 2.0 cutoffs. The 95% CIs for proportions and for area under the ROC curves (AUC) were calculated with the Clopper-Pearson method and Bootstrap analysis, respectively.

A secondary objective was to determine whether [TIMP-2]·[IGFBP7] adds significant ability in predicting positive U-Scores when combined with clinical variables. Due to the small sample size of the positive U-Score group, a least absolute shrinkage and selection operator (LASSO) regression was used to select the clinical variables to be included in the reference logistic regression model from all clinical variables significantly associated ($p < 0.1$) with positive U-Scores. The shrinkage parameter lambda was chosen based

Fig. 1. Patient cohort.

on a cross-validation procedure. Models were constructed with and without [TIMP-2]·[IGFBP7]. We calculated integrated discrimination improvement (IDI) and category-free net reclassification improvement (cfNRI) to assess the enhancement of the clinical models by the addition of [TIMP-2]·[IGFBP7]. Continuous variables were $\log_{10}$ transformed in the multivariable models.

For comparisons of baseline characteristics in end point negative and positive patients, categorical variables were analyzed using the Fisher exact or chi-square test and continuous variables using the t test and Wilcoxon rank-sum test, respectively, for normally and non-normally distributed variables. For all analyses, 2-sided p values <0.05 and one-sided p values <0.025 were considered statistically significant. Statistical analyses were performed using SAS 9.3 and R 3.0.0. Package ‘glmnet’ was used for LASSO regression [18, 19].

Results

Baseline Characteristics

Three hundred sixty-two patients had available both [TIMP-2]·[IGFBP7] and U-Score data (fig. 1), of which 347 (96%) had U-Scores of 0 and 15 (4%) had U-Scores >0 . Ten patients (3%) had U-Score values of 1, 4 (1%) had values of 2 and 1 patient had U-Score of 3 ($<1\%$; table 1).

Baseline patient characteristics are shown in table 2. Patients with a positive U-Score had significantly ($p < 0.05$) higher levels of urinary [TIMP-2]·[IGFBP7] and

microalbuminuria than those without a positive U-Score. A positive U-Score was also significantly associated with higher levels of glucose and C-reactive protein (CRP) in blood, but lower levels of serum albumin. Thirty-six percent of the patients with negative U-Score had AKI stages 1–3 within 3 days, whereas 60% of the patients with positive U-Score developed AKI.

Primary Analysis – Comparison of Urine Sediment and [TIMP-2]·[IGFBP7]

Patients were stratified into 3 groups by their [TIMP-2]·[IGFBP7] values using the validated cutoffs of 0.3 and 2.0. The proportion of patients with a positive U-Score increased across the 3 strata from 1% (95% CI 0–3) to 6% (95% CI 2–12) to 24% (95% CI 9–45) ($p < 0.001$; fig. 2). At the 0.3 cutoff, urinary [TIMP-2]·[IGFBP7] had a sensitivity of 87% (95% CI 60–98), specificity of 62% (95% CI 57–67), negative predictive value (NPV) of 99% (95% CI 97–100) and positive predictive value (PPV) of 9% (95% CI 5–15) for prediction of a positive U-Score. The AUC was 0.82 (95% CI 0.66–0.93). At the 2.0 cutoff, specificity increased to 95% (95% CI 92–97) and PPV increased to 24% (95% CI 9–45) (table 3).

Normalization by urine creatinine concentration [20] decreased the AUC to 0.77 (95% CI 0.62–0.92). However, urinary [TIMP-2]·[IGFBP7] was FDA-cleared without

Table 2. Baseline patient characteristics**a** Demographics, comorbidities, reason for presentation and outcomes

	U-Score = 0	U-Score >0	All patients	p value
All subjects	347	15	362	
Male, n (%)	229 (66)	12 (80)	241 (67)	0.402
Age, years	66 (52–75)	68 (58–70)	67 (53–74)	0.646
BMI, kg/m ²	27.4 (24.4–30.4)	25.3 (22.0–30.1)	27.3 (24.3–30.3)	0.085
Comorbidities (acute and chronic), n (%)				
Heart failure	80 (23)	1 (7)	81 (22)	0.206
Hypertension	201 (58)	9 (60)	210 (58)	>0.999
Renal insufficiency (CKD)	38 (11)	1 (7)	39 (11)	>0.999
Hypertensive nephrosclerosis	19	0	19	
Diabetic nephropathy	8	0	8	
Cardiorenal	6	0	6	
Chronic glomerulonephritis	1	0	1	
Ischemic	1	0	1	
Other ^a	3	1	4	
Diabetes	78 (22)	4 (27)	82 (23)	0.753
Peripheral arteriosclerotic disease	18 (5)	0 (0)	18 (5)	>0.999
Stroke	21 (6)	0 (0)	21 (6)	>0.999
Myocardial infarct/ACS	76 (22)	2 (13)	78 (22)	0.748
Bronchopulmonary infection	23 (7)	4 (27)	27 (7)	0.019
UTI	17 (5)	2 (13)	19 (5)	0.182
Hematocrit (anemia) (<30)	3 (1)	0 (0)	3 (1)	>0.999
Hypotension	3 (1)	0 (0)	3 (1)	>0.999
Reason for presentation (chief complaints), n (%)				0.115
Myocardial infarct/ACS	89 (26)	1 (7)	90 (25)	
Abdominal pain	23 (7)	2 (13)	25 (7)	
Cardiac decompensation	48 (14)	0 (0)	48 (13)	
Fainting/dizziness	29 (8)	1 (7)	30 (8)	
Hypertensive derailment	10 (3)	0 (0)	10 (3)	
Inflammatory diseases	82 (24)	7 (47)	89 (25)	
TAA	21 (6)	0 (0)	21 (6)	
Other	45 (13)	4 (27)	49 (14)	
Outcomes, n (%)				
AKI within 3 days	125 (36)	9 (60)	134 (37)	0.098
Admission to ICU	16 (5)	0 (0)	16 (4)	>0.999
Admission to IMC	75 (22)	4 (27)	79 (22)	0.749

^a Post calcineurin-inhibitor intake, post NSAID intake, one side renal agenesis, chronic transplant failure.

CKD = Chronic kidney disease; ACS = acute coronary syndrome; UTI = urinary tract infection; NSAID = non-steroidal anti-inflammatory drug; TAA = tachyarrhythmia absoluta; ICU = intensive care unit; IMC = intermediate care unit.

normalization, and there are no validated cutoffs for urinary [TIMP-2]·[IGFBP7] normalized by urine creatinine concentration.

Clinical Model with Categorical [TIMP-2]·[IGFBP7]

Using LASSO regression, plasma CRP was selected for the reference logistic regression model from all clinical variables in table 1 associated with a positive U-Score. When [TIMP-2]·[IGFBP7] was added to the model as a categorical variable using the 0.3 and 2.0 cutoffs, the AUC

increased significantly ($p = 0.01$) from 0.82 (95% CI 0.70–0.91) to 0.86 (95% CI 0.75–0.96) (tables 4 and 5). The adjusted OR for [TIMP-2]·[IGFBP7] between 0.3 and 2.0 was 4.72 (95% CI 0.93–23.93) and that for [TIMP-2]·[IGFBP7] >2.0 was 21.6 (95% CI 3.9–120.6), both relative to [TIMP-2]·[IGFBP7] ≤0.3 (table 4). Furthermore, addition of [TIMP-2]·[IGFBP7] to the model with CRP significantly improved model performance as assessed by IDI (0.10, 95% CI 0.03–0.25, $p = 0.001$) and cNRI (0.71, 95% CI 0.29–1.31, $p = 0.002$; table 5).

Table 2. (Continued)**b** Baseline urine and blood test results

	U-Score = 0	U-Score >0	All patients	p value
Urine tests				
[TIMP-2]·[IGFBP7], (ng/ml ²)/1,000	0.19 (0.06–0.60)	1.46 (0.66–2.46)	0.21 (0.07–0.66)	<0.001
Microalbuminuria, mg/dl	18 (5–57)	90 (30–192)	18 (5–74)	0.004
Protein-to-creatinine ratio, spot urine	0.14 (0.10–0.24)	0.37 (0.20–0.73)	0.14 (0.10–0.24)	<0.001
Urine creatinine, mg/dl	56.7 (33.9–92.3)	129 (108–157)	59.3 (34.3–98.0)	<0.001
Blood tests				
Enrollment serum creatinine, mg/dl	0.9 (0.8–1.2)	0.9 (0.8–1.6)	0.9 (0.8–1.2)	0.388
Enrollment plasma cystatin C, mg/dl	1.02 (0.85–1.40)	1.19 (1.09–1.42)	1.03 (0.85–1.40)	0.199
Leukocytes, G/l	8.0 (6.2–10.0)	9.1 (5.3–13.0)	8.0 (6.2–10.2)	0.356
Hemoglobin, g/l	139 (16)	138 (20)	139 (16)	0.782
Hematocrit, l/l	0.408 (0.044)	0.401 (0.055)	0.407 (0.044)	0.563
Platelets, G/l	217 (177–267)	194 (134–277)	216 (177–268)	0.558
CRP, mg/dl	0.4 (0.1–2.1)	6.3 (1.8–12.5)	0.5 (0.1–2.3)	<0.001
GOT, U/l	27 (22–36)	35 (16–51)	27 (22–36)	0.468
GPT, U/l	24 (17–37)	26 (15–42)	25 (17–37)	0.789
Bilirubin, mg/dl	0.5 (0.4–0.8)	0.5 (0.3–1.2)	0.5 (0.4–0.8)	0.559
Protein, g/dl	7.1 (6.7–7.4)	7.1 (6.8–7.6)	7.1 (6.7–7.4)	0.345
Albumin, g/dl	3.5 (3.2–3.8)	3.0 (2.8–3.4)	3.5 (3.1–3.8)	0.003
BNP, pg/ml	353 (77–1,589)	571 (242–3,360)	371 (80–1,650)	0.105
Glucose, mg/dl	120 (104–148)	159 (128–204)	122 (105–151)	0.002
Potassium, mmol/l	4.0 (3.7–4.2)	3.9 (3.4–4.5)	4.0 (3.7–4.2)	0.841
Chloride, mmol/l	103 (99–105)	100 (94–105)	103 (99–105)	0.187
Calcium, mmol/l	2.26 (2.18–2.35)	2.23 (2.12–2.30)	2.26 (2.18–2.35)	0.207
Phosphate, mmol/l	0.98 (0.83–1.12)	0.87 (0.65–1.06)	0.98 (0.83–1.12)	0.098
PTH, pmol/l	4.7 (3.5–7.3)	4.2 (3.3–8.1)	4.7 (3.5–7.3)	0.785
pH	7.382 (7.354–7.41)	7.386 (7.348–7.43)	7.382 (7.354–7.41)	0.659
Bicarbonate, mmol/l	24.3 (23.4–25.6)	22.6 (21.9–25.6)	24.3 (23.3–25.6)	0.149

Fig. 2. Percentage of patients with U-Scores >0 within each [TIMP-2]·[IGFBP7] stratum. 217, 120 and 25 patients had [TIMP-2]·[IGFBP7] values ≤ 0.3 , 0.3–2.0 and >2.0, respectively. The percentage (95% CI) of patients with U-Score >0 significantly ($p < 0.001$) increased across the 3 strata from 1% (95% CI 0–3) to 6% (95% CI 2–12) to 24% (95% CI 9–45).

Discussion

Urine microscopy is a useful tool to assess potential damage of the kidney and can aid in the differential diagnosis of AKI. This technique can detect glomerular hema-

turia in patients with glomerulonephritis, and the presence of white blood cell casts and sterile pyuria in urine sediment is an indicator of acute interstitial nephritis [21–23]. Urine from patients with prerenal AKI may contain hyaline casts but is commonly otherwise unremark-

Table 3. Characteristics of [TIMP-2]·[IGFBP7] at 2 cutoffs to predict positive U-Score (95% CI)

	[TIMP-2]·[IGFBP7] 0.3 cutoff, % (95% CI)	[TIMP-2]·[IGFBP7] 2.0 cutoff, % (95% CI)
Sensitivity	87 (60–98)	40 (16–68)
Specificity	62 (57–67)	95 (92–97)
NPV	99 (97–100)	97 (95–99)
PPV	9 (5–15)	24 (9–45)

Table 4. Logistic regression model with CRP alone and with CRP and categorical [TIMP-2]·[IGFBP7]. Endpoint was positive U-Scores at enrollment

Adjusted OR for	Reference model		New model	
	value (95% CI)	p value	value (95% CI)	p value
CRP	6.25 (2.77–14.11)	<0.001	4.99 (2.02–12.30)	<0.001
[TIMP-2]·[IGFBP7] >0.3 to ≤2.0 [†]	NA	NA	4.72 (0.93–23.93)	0.06
[TIMP-2]·[IGFBP7] >2.0 [†]	NA	NA	21.6 (3.9–120.6)	<0.001
AUC	0.82 (0.70–0.91)	<0.001	0.86 (0.75–0.96)	<0.001

[†] The overall p value for [TIMP-2]·[IGFBP7] is <0.001 (likelihood ratio test).

able [24]. In contrast, the typical sediment from patients with ATN contains RTE cells and casts [25, 26]. To standardize and compare urinary microscopy in patients with ATN, U-Scores were established [4, 27]. U-Scores can be used to differentiate between AKI causes and to estimate AKI severity. Perazella et al. [25] showed that U-Score ≥2 versus 1 had an OR for ATN of 74, indicating an intrarenal injury. The same authors showed that U-Score ≥3 versus 0 was associated with a 7-fold increased relative risk for worsening AKI [4].

Despite its potential benefits, urine microscopy demands immediate examination and requires substantial effort from an experienced examiner to perform. In a study of 350 urine samples, Piccoli et al. [5] determined that the average time to perform a urine sediment examination was 11.9 min. In addition, urine microscopy seems to be an ‘abandoned art’ as Fogazzi and Grignani [6] assumed in 1998. Furthermore, a loss of experience in performing urine microscopy, indicated by a reduced inter-observer agreement [28], suggests that few investigators still have the experience to perform a urine sediment examination properly.

Recently urine microscopy re-emerged in AKI diagnostics and was proposed as a renal biomarker which may be combined with novel biomarkers to improve diagnosis [3]. The few studies comparing urine sediment

Table 5. Integrated discrimination improvement, net reclassification improvement and AUC difference between model with CRP alone and with CRP and [TIMP-2]·[IGFBP7]

	Value (95% CI)	p value
IDI	0.10 (0.03 to 0.25)	0.001
IDI_event	0.09 (0.03 to 0.24)	0.001
IDI_non	0.004 (0.001 to 0.01)	0.001
cfNRI	0.71 (0.29 to 1.31)	0.002
cfNRI_event	0.47 (–0.33 to 1.00)	0.12
cfNRI_non	0.24 (0.16 to 0.92)	<0.001
AUC_ref_model	0.82 (0.70 to 0.91)	<0.001
AUC_new_model	0.86 (0.75 to 0.96)	<0.001
Increase_in_AUC	0.05 (0.006 to 0.11)	0.01

examination with novel renal biomarkers, such as neutrophil gelatinase-associated lipocalin (NGAL), have shown that urine sediment findings and the novel biomarkers were complimentary in AKI assessment [16, 29]. For example, in a study of 363 ED patients, it was shown that the novel biomarkers are more sensitive but less specific for AKI than urine sediment findings; urine microscopy had a specificity of 91% but a sensitivity of only 22% for AKI, whereas NGAL had a specificity of 65% and sensitivity of 65% [14]. The complementary

sensitivity and specificity of urine sediment analysis and novel biomarkers, along with the additional information urine sediment provides about the cause of AKI, suggest that combining these diagnostic tests may improve AKI diagnosis.

Urinary [TIMP-2]·[IGFBP7] has been cleared recently by the FDA as the first biomarker test for risk assessment of AKI [13]. TIMP-2 and IGFBP7 are renal stress biomarkers, which are involved in cell cycle arrest [8]. For the first time, we examined urine sediment in combination with urinary [TIMP-2]·[IGFBP7] in ED patients at risk for AKI. In our study, only 1.6% of the patients had a positive U-Score, implying that urine microscopy was unnecessary for most patients. Based on the time measurements of Piccoli et al. [5], in our study cohort of 397 patients it would have taken 78.74 h to perform the urine sediment examinations. Assuming an 8-hour workday, it would have been 9.84 days or 2 weeks for one examiner to conduct this analysis for the entire cohort, but in routine clinical practice urine microscopy would not have been performed for all patients.

To deploy the urine scoring system more effectively, it is necessary to select patients who are likely to show a U-Score >0. A stepwise approach in the utilization of diagnostic tests in AKI was already proposed [30]. In our cohort of ED patients 99% of patients with urinary [TIMP-2]·[IGFBP7] levels ≤ 0.3 at time of enrollment had a negative U-Score (NPV). Thus, our study suggests that a urinary [TIMP-2]·[IGFBP7] cutoff of 0.3 can be used to rule out patients for a positive U-Score. Furthermore, a urinary [TIMP-2]·[IGFBP7] cutoff of 2.0 identified patients with significantly increased risk of a positive U-Score (6-fold greater than the pre-test probability, PPV = 24%).

In this study, patients with a positive U-Score also had significantly higher levels of microalbuminuria, protein-to-creatinine ratio in spot urine and CRP. These factors are well known to be involved in AKI and ATN [15, 31]. Urinary [TIMP-2]·[IGFBP7] remained a significant predictor of a positive U-Score when adjusted for CRP in a multivariable model (adjusted OR 21.6 (95% CI 3.9–120.6) for [TIMP-2]·[IGFBP7] >2.0 relative to [TIMP-2]·[IGFBP7] ≤ 0.3 ; table 4).

Conclusions

In conclusion, urinary [TIMP-2]·[IGFBP7] was predictive of a positive U-Score and, therefore, may provide a means to more efficiently utilize urine microscopy,

which is a helpful tool for assessment of AKI cause and severity. We propose a stepwise approach in which a urinary [TIMP-2]·[IGFBP7] measurement is used to stratify patients for likelihood of having positive urine findings. In this way, the use of urinary [TIMP-2]·[IGFBP7] could help save time and personnel resources by avoiding unnecessary urine sediment examination.

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Author Contributions

M.K., M.D.A. and M.B.S. designed the study. M.K., M.B.S. and J.S. had full access to all data and take responsibility for the integrity of the data and the accuracy of the data analysis. Data collection: M.K. and C.W. enrolled subjects, M.K., C.W. and D.B. gathered data. All authors reviewed the data and participated in discussions related to interpretation. M.B.S. and M.K. wrote the first draft of the manuscript. All authors contributed to editing the manuscript and approved the final draft.

Neither this manuscript nor substantial parts of it are under consideration for publication elsewhere, have been published nor made available elsewhere in a manner that could be construed as a prior or duplicate publication of the same content. The results presented in this paper have not been published previously in whole or part, except in abstract form.

Disclosure Statement

M.K. received lecture honoraria by Abbott, Roche and Astute Medical. M.D.A. received lecture honoraria by Abbott and Roche.

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