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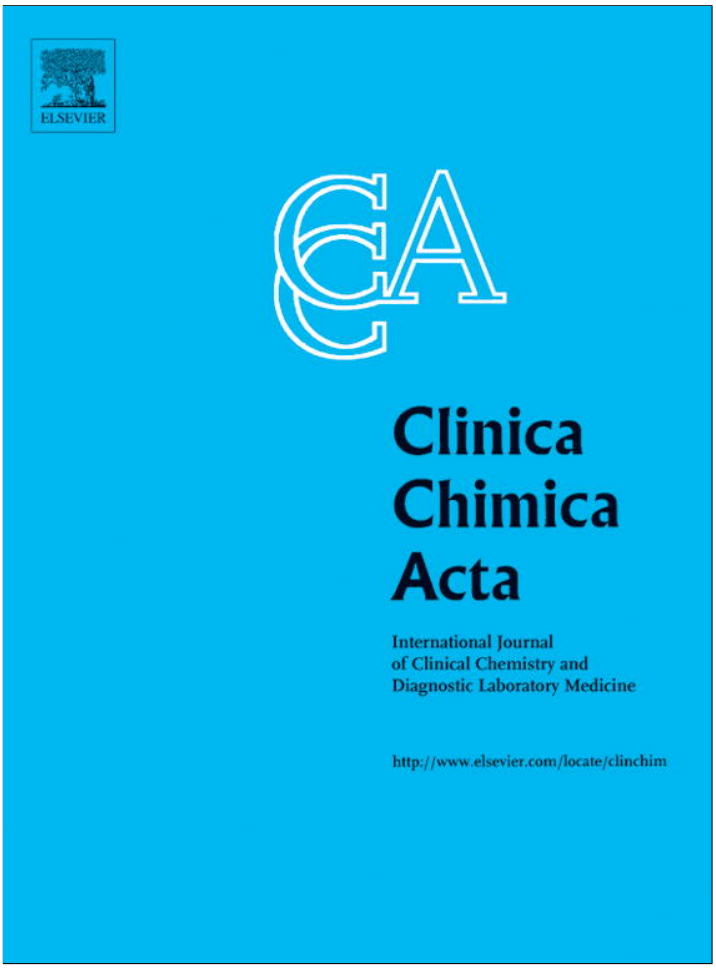

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Reference intervals of urinary acute kidney injury (AKI) markers [IGFBP7]·[TIMP2] in apparently healthy subjects and chronic comorbid subjects without AKI

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ABSTRACT

Background: Insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2) have demonstrated significantly improved diagnostic performance in assessing risk for acute kidney injury (AKI) compared with existing biomarkers. We present the findings of a multi-site trial to determine the reference intervals for these biomarkers in apparently healthy adults and those with stable chronic morbid conditions without AKI.

Methods: A urine specimen was collected from apparently healthy subjects (N = 378) and subjects with at least one stable chronic morbidity (N = 372). Specimens were kept frozen until analysis with the NephroCheck® Test (Astute Medical). The test is comprised of fluorescence immunoassays for IGFBP7 and TIMP-2 and is used with the Astute140® Meter which quantifies the concentration of each biomarker. The meter multiplies the concentrations of IGFBP7 and TIMP-2 and displays the result as a numerical value ([IGFBP7]·[TIMP-2]) expressed in $(ng/ml)^2/1000$ which is called the AKIRisk™ Score.

Results: The reference intervals (inner 95%) for [IGFBP7]·[TIMP-2] in all subjects (N = 750), apparently healthy subjects, and subjects with stable chronic morbidities were 0.04–2.22, 0.04–2.25, and 0.05–2.20 $(ng/ml)^2/1000$ respectively. There was no statistical difference between reference intervals for apparently healthy and chronic stable morbid cohorts (p = 0.42).

Conclusions: Our investigation showed that urine [IGFBP7]·[TIMP-2] values were not elevated in patients with stable chronic morbidities who did not have AKI.

1. Introduction

Acute kidney injury (AKI) is a disease with multiple etiologies that lacks an effective specific treatment in current clinical practice [1]. Various exposures (e.g., infections, medications, toxins) can cause kidney damage leading to AKI, which results in rapid loss of function. AKI can lead to a temporary or permanent loss of kidney function or even death. AKI substantially increases mortality, morbidity, length of hospitalization, adverse long term health consequences, and the cost of healthcare [2,3]. The annual death rate from AKI is higher than breast cancer, prostate cancer, and heart failure combined [4]. Irreversible kidney failure might be preventable if biomarkers can identify AKI early in the disease process [5–7]. Ideally, AKI biomarkers should be sensitive and specific. In order to change patient outcome they should be able to identify AKI early in the disease process in an easily accessible matrix (e.g., urine). In reality, it is difficult to find such biomarkers and no previously described biomarkers possess all of these characteristics [8].

Recently, Kashani et al. reported insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-

Abbreviations: AKI, acute kidney injury; IGFBP7, insulin-like growth factor-binding protein 7;; KDIGO, kidney disease improving global outcomes; NGAL, neutrophil gelatinase-associated lipocalin; TIMP-2, tissue inhibitor of metalloproteinases-2.

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2), as two novel biomarkers for AKI [9]. These investigators demonstrated that elevation of IGFBP7 and TIMP-2 are predictive of the development of moderate to severe AKI [kidney disease improving global outcomes (KDIGO) stage 2 to 3] within 12 h of specimen collection [10]. These 2 biomarkers performed better than previously identified biomarkers such as kidney injury marker-1 (KIM-1), neutrophil gelatinase associated lipocalin (NGAL), cystatin-C, interleukin-18, pi-glutathione S-transferase, and liver fatty acid-binding protein. A subsequent multi-site international study (Topaz) using identical entry criteria which also used clinical adjudication for AKI endpoints, confirmed that these biomarkers can identify patients at high risk for imminent AKI [11].

IGFBP7, also known as Mac25, tumor adhesion factor (TAF), prostacyclin stimulating factor (PSF), and IGFBP-rP1 belongs to the IGFBP family. IGFBP7 has been hypothesized to have a significant biological role in cell proliferation, apoptosis, and senescence [12–14]. TIMP-2 is a member of the matrix metalloproteinase family that has been shown to mediate both tissue development and remodeling [15]. Both IGFBP7 and TIMP-2 have been implicated in G_1 cell-cycle arrest [16–18]. For short periods of time, cell cycle arrest is likely protective, preventing cells from entering cell cycle during periods of imminent or current injury [19]. However, when cell cycle arrest is prolonged, cells can transition to a fibrosis phenotype. Studies of AKI demonstrated the role of prolonged cell cycle arrest in the transition of AKI to CKD [20]. Early on, activation of these cell cycle arrest markers function as an alarm signal, indicating something is wrong. The time window for cell cycle arrest activation to be protective is likely brief, and sustained cell cycle arrest can be maladaptive. An analogy would be cortisol activation during stress. In the short-term, this response is protective, but sustained cortisol stimulation is linked to increased morbidity. In the case of cell-cycle arrest, the harm may come from cell senescence (a phenotype in which the cells can never re-enter cell division and in which fibroblasts rather than new epithelial cells are attracted to close gaps in renal tubular epithelium) which may lead to CKD.

This is the first report of the observed values (reference interval) of [IGFBP7]·[TIMP-2] in urine where a large group of subjects including apparently healthy individuals and individuals with stable chronic morbidities were studied in order to determine how these biomarkers varied in different patient populations that were not acutely ill and did not have signs or clinical findings of AKI.

2. Materials and methods

2.1. Study design and study participant selection

The protocols for this investigation were approved by investigational review boards/ethics committees as required by each participating institution. All subjects provided written informed consent. Subjects of ≥21 y age, who provided written informed consent for the study participation, and met the morbidity criteria (Table 1) were selected in the stable chronic morbidity cohort. A listing of the morbid conditions and number of subjects with each condition is shown in Table 1 of Chindarkar et al. [21]. For apparently healthy subjects, individuals of ≥21 y, who provided written informed consent for study participation, and met the healthy criteria (Table 3 of Chindarkar et al. [21]), were selected for this cohort. The urine specimens were collected at 6 geographically diverse sites (Rochester, NY; Dallas, TX; Gresham, OR; Springfield, MO; Layton, UT; Peoria, AZ). The specimen collected from each subject was split into three aliquots and frozen (−80 °C) within 120 min of collection. The frozen specimens were shipped to 3 different testing sites for analysis (University of California at San Diego; University of Louisville; ARUP Laboratories). Specimens were collected over the time frame of April 2012 to November 2012.

Table 1
Inclusion and exclusion criteria for chronic stable morbid subjects.
An individual of age ≥21 y and with any one or more of the following chronic, stable, morbid conditions (in addition to meeting the exclusion criteria), was classified as 'chronic, stable, morbidity subject'.

Inclusion criteria (*morbidities*)	
— Hypertension	— Bladder, prostate, or renal cell cancer
— Osteoarthritis	— Chronic pancreatitis
— Coronary artery disease	— Chronic renal insufficiency
— Gout	— Cancer affecting kidney function
— Congestive heart failure	— Other kidney disease including polycystic kidney disease
— Diabetes mellitus (type 1 or 2)	*Exclusion criteria*
— Arrhythmia (atrial fibrillation, heart block, ventricular tachycardia)	— Subject with any known or suspected acute illness or condition - including acute infections — at the time of enrollment or within the previous 30 days
— Hyperlipidemia (includes hypercholesterolemia)	— Subject with any new onset or unstable morbidities
— Hyper- or hypothyroidism	— Subject with any trauma-related surgery within the last 6 months
— Chronic obstructive pulmonary disease (including emphysema chronic bronchitis, and asthma)	— Subject with any surgery, hospitalization or institutionalization (such as in a nursing home) during the previous 3 months
— Neuromuscular disease	— Subject who received any blood product transfusion within the previous 2 months
— Immunocompromised	— Subject who was a pregnant woman or child
— Liver cirrhosis	— Subject who was a prisoner or institutionalized individual
— Rheumatoid arthritis	— Subject who has previously enrolled in this study
— Hepatic failure	
— Systemic lupus erythematosus	
— Inflammatory bowel disease (including Crohn's disease and ulcerative colitis)	
— Chronic coagulation abnormality	
— Any active cancer	

2.2. Demographic characteristics

A total of 379 and 373 subjects were enrolled in the apparently healthy and stable chronic morbidity cohorts respectively (Fig. 1). One subject was excluded from each cohort to give a total of 750 subjects. The two subjects were excluded because specimens from these subjects were processed outside the allowable time window of 120 min of collection. Demographic characteristics of the 750 subjects selected for this study are described in the Table 2. The target demographic distribution used in this study is provided in the Table 3 of Chindarkar et al. [21]. This distribution was targeted to reflect the general demographic data for United States intensive care units.

2.3. Biomarker measurement

The NephroCheck® Test is an FDA cleared [22] in-vitro diagnostic test that quantitatively measures IGFBP7 and TIMP-2 in human urine on the Astute140® Meter, a bench/table-top analyzer [23]. It is a single-use cartridge comprised of sandwich immunoassays for IGFBP7 and TIMP-2 on a membrane test strip enclosed in a plastic housing. The Astute140® Meter converts the fluorescent signal from each of the 2 immunoassays (IGFBP7 and TIMP-2) contained within the NephroCheck® Test cartridge into a single numerical result called the AKIRisk™ Score. The AKIRisk™ Score i.e. [IGFBP7]·[TIMP-2] is calculated as the product of the measured concentrations of the 2 biomarkers, IGFBP7 and TIMP-2 (in $(ng/ml)^2$), divided by 1000. The test result ([IGFBP7]·[TIMP-2]) is displayed without units. The limit of blank, limit of detection, limit of quantitation and reportable range for [IGFBP7]·[TIMP-2] were 0.0002, 0.002, 0.002, and 0.04–10.0 respectively [23]. Concentrations of IGFBP7 and TIMP-2 are traceable to reference standards as described by ISO 17511 [5]. Each single use cartridge includes a built in positive and negative control. If the automatic check of these internal controls shows that the

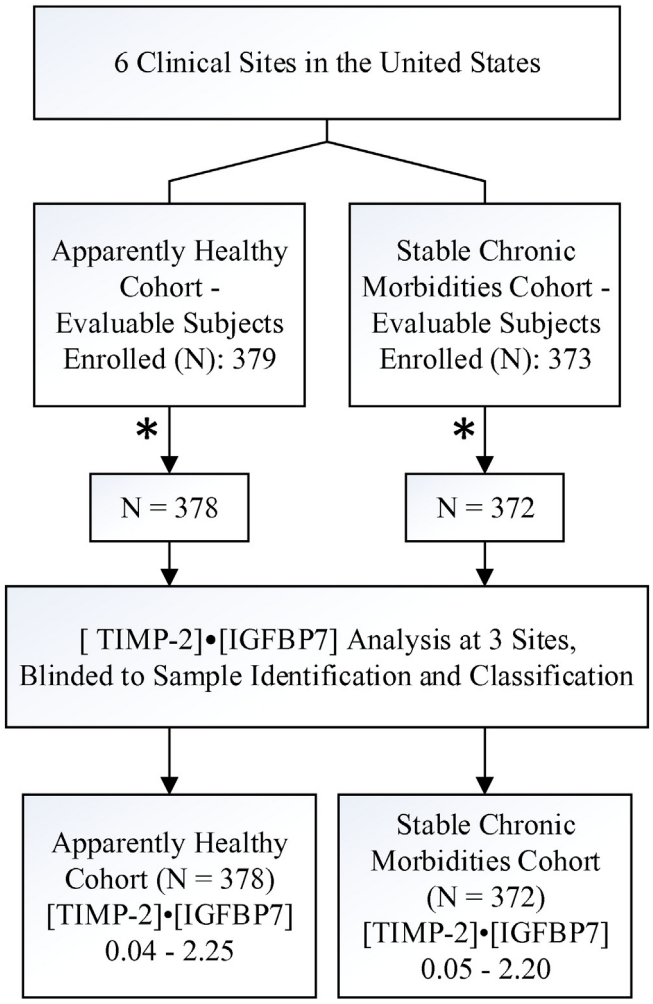

Fig. 1. Reference interval subjects selection, specimen collection, and analysis. All selected specimens were subjected to analysis by NephroCheck® Test and Astute140® Meter. (* One subject was excluded for protocol deviation).

Table 2
Demographic characteristics of apparently healthy and chronic stable morbidity cohorts.

	Apparently healthy cohort (N = 378)		Chronic stable morbidity cohort (N = 372)	
Variable/stratum	N, mean, or median	%, SD, or IQR	N, mean, or median	%, SD, or IQR
Sex				
Female	191	(50.5)	191	(51.3)
Male	187	(49.5)	181	(48.7)
Race				
American Indian	3	(0.8)	6	(1.6)
Asian	9	(2.4)	10	(2.7)
Black/African	43	(11.4)	43	(11.6)
Amer.				
Native	1	(0.3)	3	(0.8)
Hawaiian				
Caucasian	313	(82.8)	300	(80.6)
Unknown	0	(0.0)	0	(0.0)
Other	9	(2.4)	10	(2.7)
Ethnicity				
Hispanic	43	(11.4)	33	(8.9)
Non-Hispanic	335	(88.6)	339	(91.1)
Age (years)				
Mean (SD)	54	(17.3)	63	(14.7)
Median (IQR)	56	(40–68)	65	(53–75)
Height (cm)				
Mean (SD)	169.5	(9.87)	168.2	(10.17)
Median (IQR)	170.0	(162.6–177.8)	167.6	(160.0–175.3)
Weight (kg)				
Mean (SD)	78.8	(18.74)	87.0	(21.93)
Median (IQR)	77.0	(65.6–86.9)	84.3	(71.6–99.2)
BMI (kg/m^2)				
Mean (SD)	27.5	(5.87)	30.8	(7.02)
Median (IQR)	26.8	(23.3–29.8)	29.8	(26.2–34.5)

control value results are not within pre-defined limits, the meter will display an error message and the test result will not be reported. In addition, external liquid and electronic quality controls were used to ensure proper functioning of each Astute140® Meter used for specimen analysis. Two external liquid controls (low and high concentrations) were analyzed once on each meter when they were initially put into use to verify test performance and operator proficiency. IGFBP7 and TIMP-2 concentrations in the high control were 419.3 and 127.6 ng/ml respectively while in low control they were 61.2 and 3.1 ng/ml respectively. Liquid control results were acceptable if they were within ±2 SD of the expected concentrations. An external electronic control was run on each meter every day prior to the specimen analysis. The external electronic control procedure verifies the calibration of the Astute140® Meter, including the positioning system, optical system and other internal systems of the meter to confirm that the meter is functioning properly.

Frozen specimens were thawed at room temperature (18–25 °C) in a water bath for a thawing time not exceeding 20 min. Once the specimen was thawed, it was gently inverted 1–2 times to ensure uniform mixing and was immediately tested. The specimen was loaded into a NephroCheck® Test cartridge within one hour of placing the specimen into the water bath. A 100 μL aliquot of the buffer supplied with the assay kit was added to a labeled fluorescent conjugate vial followed by addition of a 100 μl aliquot of thawed urine specimen. The solution was mixed gently. The mixture (100 μl) was then loaded onto a NephroCheck® Test cartridge. The test cartridge was loaded onto the Astute140® Meter which provides results in 20 min. [IGFBP7]·[TIMP-2] value was displayed on the Astute140® Meter screen after the NephroCheck® Test procedure was completed.

2.4. Statistics

Reference intervals were defined as the inner 95th percentile of the rank ordered data [24,25]. The intervals were determined by sorting NephroCheck® Test results (from 3 testing sites) from low to high, and then using the CEIL function in SAS ver 9.2 [SAS Institute Inc.] to determine the rank corresponding the percentile of interest [rank for the low end of reference interval = CEIL (0.025 * N), rank for the high end of reference interval = CEIL (0.975 * N), where N = total number of specimens]. The test values corresponding to the ranks are the values for the reference interval. The distributions of [IGFBP7]·[TIMP-2] values from the apparently healthy and stable chronic morbidity cohorts were compared using the Kolmogorov–Smirnov test. We used log-linear analysis to test null hypotheses of independence between the reference interval ([IGFBP7]·[TIMP-2] values below, within, and above the inner 95%), study cohort (apparently healthy or stable chronic morbidity), and sex [26]. Because there were multiple [IGFBP7]·[TIMP-2] test results per sample, a permutation test was used to calculate p-values. Two-sided $p < 0.05$ were considered statistically significant. Statistical tests were performed using R 3.0.1 [27]. To assess associations between mean [IGFBP7]·[TIMP-2] values and age, we calculated Spearman's rank correlation coefficient (r_s) within each cohort. For the precision studies, the method described by Rodbard et al. was used to determine the CV within each tertile [28].

Table 3
Rank ordered percentile for [IGFBP7]·[TIMP-2] values in all 750 subjects.

Population	Male/female	Subjects	Specimens[a]	Reference interval[b]	Percentile								
					2.5	5	10	25	50	75	90	95	97.5
All	Both	750	2246[c]	0.04–2.22	0.04	0.05	0.07	0.14	0.33	0.73	1.29	1.77	2.22
Apparently healthy subjects	Both	378	1132	0.04–2.25	0.04	0.05	0.07	0.14	0.32	0.74	1.29	1.85	2.25
Chronic stable morbidity subjects	Both	372	1114	0.05–2.20	0.05	0.06	0.07	0.13	0.34	0.72	1.29	1.75	2.20
Apparently healthy subjects	Female	191	573	0.04–2.24	0.04	0.04	0.06	0.12	0.29	0.68	1.28	1.79	2.24
	Male	187	559	0.04–2.25	0.04	0.05	0.09	0.17	0.36	0.77	1.33	1.88	2.25
Chronic stable morbidity subjects	Female	191	573	0.04–2.15	0.04	0.05	0.06	0.13	0.36	0.76	1.29	1.76	2.15
	Male	181	541	0.06–2.23	0.06	0.07	0.08	0.13	0.33	0.70	1.24	1.69	2.23

Note: 48% of the [IGFBP7]·[TIMP-2] values were ≤0.3 and 96% were ≤2.0). [IGFBP7]·[TIMP-2] are expressed in $(ng/ml)^2/1000$

[a] A specimen from each subject was divided into three aliquots. Aliquots were tested at three independent hospital laboratories.

[b] Based on 2.5% and 97.5% values in sets of at least 120 subjects.

[c] Total number of specimens was 2250 (750 × 3) but 4 specimen were not included due to 2 specimen handling protocol deviations, 1 invalid result that was erroneously not retested as specified in the protocol, and 1 shipment error.

3. Results and discussion

Of the 750 subjects selected for this study, 378 were categorized as apparently healthy while 372 were categorized as subjects with chronic stable morbidities but no AKI. The distributions of all [IGFBP7]·[TIMP-2] values from the apparently healthy and chronic stable morbidity cohorts are shown in Fig. 2. There was no statistically significant difference between the distributions of these cohorts ($p = 0.62$). The overall reference interval of [IGFBP7]·[TIMP-2] for the entire population studied ($N = 750$) was 0.04–2.22 (Table 3). Reference intervals for apparently healthy ($N = 378$) and chronic stable morbid ($N = 372$) subjects were 0.04–2.25 and 0.05–2.20, respectively. There was no statistically significant difference between these reference intervals ($p = 0.42$). Further stratification of the cohorts by sex (male or female) did not significantly affect the reference interval ($p = 0.14$ for the test of dependence of the reference interval on cohort and sex). Within the apparently healthy cohort, the reference intervals for males and females were 0.04–2.25 ($N = 187$) and 0.04–2.24 ($N = 191$), respectively. Within the chronic stable morbidity cohort, the reference intervals for males and females were 0.06–2.23 ($N = 181$) and 0.04–2.15 ($N = 191$), respectively. As shown in Fig. 3, there was a weak inverse correlation between mean [IGFBP7]·[TIMP-2] value and age in both cohorts ($r_s = -0.13$, $p = 0.014$, within the apparently healthy cohort, and $r_s = -0.11$, $p = 0.038$, within the chronic stable morbidity cohort).

To demonstrate the consistency of results obtained at three different testing laboratories, we calculated the inter-laboratory precision (% CV) of [IGFBP7]·[TIMP-2] measurements (Table 4). A urine specimen from each qualifying subject was analyzed in singlicate at the three sites. We used the average of all [IGFBP7]·[TIMP-2] values for each subject and split the concentration range into tertiles in order to demonstrate precision at the low, medium and high concentrations of test results. The inter-laboratory CV was 10% or less within each tertile.

Serum creatinine, serum urea, and urine output are conventional biomarkers of kidney function [29–32] and as such have limitations for prognosticating risk of renal injury. By definition, loss of kidney function indicates renal injury, which causes the end products of nitrogen metabolism (e.g. urea and creatinine) to accumulate in circulation. When measured in a serial manner, small increases of creatinine, ≥0.3 mg/dl over 48 h, define AKI [10], but at this point renal injury has occurred. In comparison, [IGFBP7]·[TIMP-2] are not indicators of renal function, rather they indicate early signs of renal stress, thus they appear to be fundamentally different than renal function biomarkers. [IGFBP7]·[TIMP-2] are more appropriately used to evaluate risk of renal injury as opposed to documenting decline in function.

Among the previously described biomarkers for AKI, NGAL has been extensively investigated [33–36]. Since its discovery [36], NGAL has shown better predictive and diagnostic value compared to creatinine and has been considered a promising biomarker of AKI. NGAL showed excellent sensitivity and specificity in pediatric patients in predicting AKI after cardiac surgery [35]. However, a primary limitation of this biomarker is that its concentration may rise in the setting of chronic and acute inflammatory conditions frequently encountered in the ICU patients [33,34]. NGAL is also expressed by tissues other than the kidney [37].

For patients at risk for AKI in the immediate future, the goal is to detect AKI as early as possible. Early detection gives clinicians time to consider various treatment and/or management options. If a patient is at high risk for AKI, a more conservative treatment approach can be

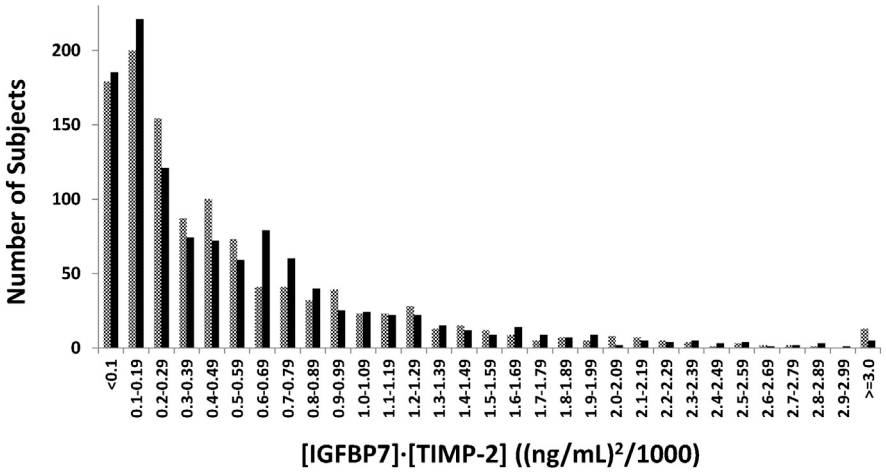

Fig. 2. [IGFBP7]·[TIMP-2] distribution in apparently healthy (gray bars) and chronic morbid (no AKI) subjects (solid bars).

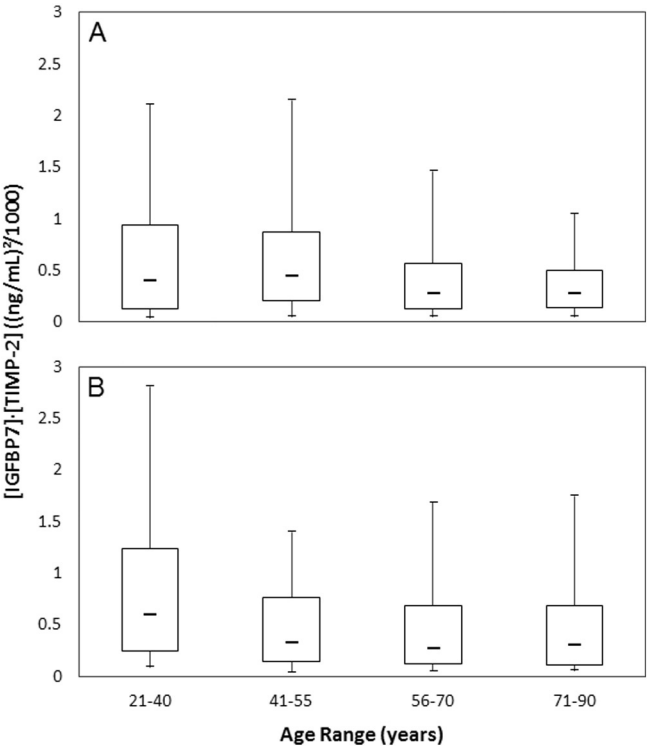

Fig. 3. [IGFBP7]·[TIMP-2] values by age range for (A) apparently healthy subjects and (B) subjects with chronic stable morbidities. Boxes and whiskers show interquartile ranges and 5th to 95th percentiles, respectively. Horizontal dashes within the boxes show median values.

taken and the patient can be spared from potential nephrotoxic agents. Studies from Kashani et al. [9] and Bihorac et al. [11] demonstrated that early detection of AKI is possible with the help of IGFBP7 and TIMP-2. These authors reported that elevated concentrations of IGFBP7 and TIMP-2 predict AKI within 12 h of becoming elevated. The study by Kashani and colleagues involved 744 critically ill patients, including those with acute illnesses such as sepsis (but without a diagnosis of AKI at enrollment), to see how these 2 biomarkers perform compared to other markers. All patients enrolled in this study were in the intensive care unit and expected to be there for at least 48 h. Comparison of IGFBP7 and TIMP-2 with existing biomarkers based on area under receiver-operating characteristic curve (AUC) revealed that together IGFBP7 and TIMP-2 (AUC of 0.8) performed significantly better than other biomarkers. It was observed that the risk of AKI within 12 h increased sharply when [IGFBP7]·[TIMP-2] values were >0.3 and quintupled when >2.0 [9].

Recently Hoste et al. verified 0.3 and 2.0 cutoff values for [IGFBP7]·[TIMP-2] in 154 critically ill patients [6]. A high sensitivity (89%) cutoff of 0.3 was chosen in order to identify any patients who were at increased risk for AKI within 12 h of assessment. This cutoff had a 97% negative predictive value and indicated a low probability of AKI when the patient tested at or below this cutoff. The second cutoff of 2.0 had high specificity (>90%) and indicates patients with a very high risk of developing AKI within 12 h. The authors report that the results agree with previous investigation [9] in terms of predicting the AKI within 12 h of the specimen collection.

Table 4
Inter-laboratory percent CV of [IGFBP7]·[TIMP-2] values obtained from 3 independent hospital laboratories on 750 subjects. A specimen was collected from each subject and divided into three aliquots. Each testing site received an aliquot from each subject. The average [IGFBP7]·[TIMP-2] values were rank ordered and divided into tertiles. The inter-laboratory CV within each tertile is shown below.

Tertile	[IGFBP7]·[TIMP-2] in (ng/ml)^2/1000	% CV
1st	0.04–0.18	9
2nd	0.19–0.57	10
3rd	0.58–7.18	9

Bihorac et al. used a cutoff of 0.3 to identify patients at a risk of developing AKI in 420 critically ill patients [15]. The 0.3 cutoff had a sensitivity and specificity of 92% and 46%, respectively. Patients developing AKI within 12 h had a median (interquartile range [IQR]) urinary [IGFBP7]·[TIMP-2] of 1.6 (0.7–2.8) compared with those without AKI in which the median (IQR) urinary [IGFBP7]·[TIMP-2] was 0.3 (0.2–0.8). The authors demonstrated that critically ill patients with urinary [IGFBP7]·[TIMP-2] >0.3 had 7 times the risk for AKI as compared with critically ill patients with a test result <0.3.

The median and IQR reported by Bihorac et al. for critically ill patients without AKI is similar to the median (50th percentile) and IQR (25th to 75th percentiles) for apparently healthy subjects (0.32 (0.14–0.74)) and subjects with stable chronic morbidities (0.34 (0.13–0.72)) shown in Table 3. These data confirm that [IGFBP7]·[TIMP-2] values generally are not elevated in subjects with common chronic morbidities or in critically ill patients with acute conditions other than AKI. Significant elevations in [IGFBP7]·[TIMP-2] values have only been observed in patients who have or subsequently developed AKI [5,6,9,11]. Although [IGFBP7]·[TIMP-2] values are significantly increased in AKI patients, reference intervals in apparently healthy and stable chronic morbid subjects overlap with the values obtained from AKI patients. Reference interval data show that overall, 50% of the population lies >0.33 and the rest of the [IGFBP7]·[TIMP-2] values are ≤0.3 (Table 3). As such, the [IGFBP7]·[TIMP-2] values obtained in critically ill patients should only be used in conjunction with patient condition and clinical signs and symptoms. This test was developed to assess risk of AKI and not intended as a sole indicator for the diagnosis of AKI. The primary cutoff at 0.3 was selected to have high sensitivity to detect the majority of patients at risk for AKI, with a specificity near 50%, which is considered acceptable for the intended use of the test [6]. Our results show this cutoff falls in the middle of the reference range, consistent with the specificity reported previously in the intended use population [6,11]. Importantly, this test provides a quantitative result and it is clear from prior work [3,9] that AKI risk increases with increasing concentrations of [IGFBP7]·[TIMP-2]. A secondary, high specificity (near 95%) cutoff at 2.0 has been reported previously from studies of critically ill patients [6,11]. Our results show the high end of the reference range is nearly identical to this secondary cutoff value, consistent with the specificity previously reported [6,11]. The KDIGO Guideline recommends kidney sparing management strategies based on a patient's risk for AKI [10]. Patients with higher [IGFBP7]·[TIMP-2] values and the appropriate clinical presentation are candidates for these management strategies including increased surveillance, interventions, and therapy adjustments to further minimize potential for renal injury.

In summary, the overall reference interval for [IGFBP7]·[TIMP-2] in urine is 0.04–2.22. Compared with apparently healthy subjects, [IGFBP7]·[TIMP-2] values were not elevated in stable chronic morbid subjects. This is in contrast with reports for other biomarkers of AKI such as NGAL, KIM-1, and IL-18 which are elevated in a variety of conditions such as liver injury [38], chronic kidney injury [39], and a variety of other conditions [40], respectively. The cutoff for [IGFBP7]·[TIMP-2] at 0.3 falls near the middle of the reference interval, consistent with the specificity previously validated in critically ill patients. Our data also confirm that the previously reported high specificity cutoff of 2.0 has little overlap with the reference interval and higher values of [IGFBP7]·[TIMP-2] are therefore indicative of higher risk of impending AKI.

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