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RESEARCH ARTICLE

Urinary α-GST and π-GST for prediction of dialysis requirement or in-hospital death in established acute kidney injury

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Abstract

Context: Urinary α-glutathione S-transferase (α-GST) and π-glutathione S-transferase (π-GST) are promising proximal and distal tubular leakage markers for early detection of acute kidney injury (AKI).

Objective: To examine the performance of these markers for predicting the composite of dialysis requirement or in-hospital death in patients with an established diagnosis of AKI.

Materials and methods: Prospective cohort study of 245 adults with AKI. A single urinary α-GST and π-GST measurement was obtained at time of nephrology consultation.

Results: Overall, urinary π-GST performed better than α-GST for prediction of dialysis requirement (AUC 0.59 vs. 0.56), and the composite outcome (AUC 0.58 vs. 0.56). In subgroup analyses, π-GST displayed better discrimination for prediction of dialysis requirement in patients with baseline eGFR <60 mL/min/1.73 m^2 (AUC 0.61) and oliguria (AUC 0.72). Similarly, α-GST performed better in patients with stage-1 (AUC 0.66) and stage-2 AKI (AUC 0.80).

Conclusions: In patients with an established diagnosis of AKI, a single urinary π-GST measurement performed better than α-GST at predicting dialysis requirement or death, but neither marker had good prognostic discrimination.

Keywords: Acute kidney injury, acute renal failure, biomarker, glutathione S-transferase, epidemiology, prognosis

Introduction

Acute kidney injury (AKI) is a common complication in hospitalized patients (Hou et al. 1983, Levy et al. 1996, Nash et al. 2002, Liangos et al. 2006), and is associated with increased morbidity, mortality, and healthcare expenditures (Liangos et al. 2006, Chertow et al. 2005). Early diagnosis of AKI and risk stratification might allow timely nephrology consultation and provision of supportive care, which in turn might decrease morbidity and mortality. Serum creatinine is the currently accepted glomerular filtration rate (GFR) marker used to diagnose and stage AKI (Bellomo et al. 2004, Mehta et al. 2007). However, this marker is affected by several non-GFR determinants in AKI (Moran and Myers 1985, Star 1998, Bonventre and Weinberg 2003, Rosner and Okusa 2006).

In recent years, a number of studies have evaluated the potential value of several new markers for early detection of AKI and prediction of adverse outcomes, but have demonstrated variable performance (Herget-Rosenthal et al. 2004, Liangos et al. 2007, Coca et al. 2008, Perianayagam et al. 2009, Haase et al. 2009). Among these potential new markers, two subtypes of the glutathione S-transferase (GST) enzyme have been considered (McMahon et al.). These are soluble cytosolic enzymes that play a role in detoxification processes (Mannervik 1985, Sundberg et al. 1994a, Aliya et al. 2003). The kidney has two cytosolic soluble GST subtypes, α-GST and π-GST, which are

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located in different segments of the nephron. α-GST has specificity for the proximal tubule, and π-GST for the distal tubule (Feinfeld et al. 1977, Harrison et al. 1989, Campbell et al. 1991). These molecules are classified as tubular leakage markers whereby tubular injury results in the leakage of cellular content into the urine, including α-GST and π-GST. Experimental studies support the claim that elevated urinary α-GST levels indicate proximal tubular injury whereas elevated π-GST levels are more specific for a distal tubular injury (Dieterle and Sistare 2010).

Several relatively small cohort studies have evaluated the potential role of urinary α-GST and π-GST for the early detection of AKI (Eger et al. 1997, Boldt et al. 2003, Westhuyzen et al. 2003, da Silva Magro and de Fatima Fernandes Vattimo 2004, Eijkenboom et al. 2005, Yavuz et al. 2009, Koyner et al. 2010, Walshe et al. 2009) and for prediction of its severity (Herget-Rosenthal et al. 2004, Koyner et al. 2010). In the present study, we explore the potential usefulness of urinary α-GST and π-GST measured at the time of nephrology consultation, for predicting dialysis requirement and in-hospital mortality in a large cohort of hospitalized patients with an established diagnosis of AKI.

Patients & methods

Study design and setting

This was a hospital-based prospective cohort study of adults with AKI conducted at two acute care facilities located in Boston, Massachusetts. All consecutive patients with an established diagnosis of AKI, in whom nephrology consultation was requested, were eligible for study enrolment. AKI was defined as an increase in serum creatinine by 0.5, 1.0 or 1.5 mg/dL from a baseline level of ≤1.9, 2.0–4.9, and ≥5.0 mg/dL, respectively (Hou et al. 1983). This definition was adopted prior to the development of the more recent AKI network consensus definition (Mehta et al. 2007).

Exclusion criteria were age less than 18 years, pregnancy, end-stage renal disease on maintenance dialysis, organ transplantation within the previous year, and acute obstructive uropathy. Patients receiving palliative or terminal care were also excluded. Written informed consent was obtained from study participants or next of kin. The institutional review board of each facility approved the study protocol.

Data collection

Hospital records were reviewed prospectively to retrieve information on baseline demographic characteristics, coexisting conditions, and kidney-related variables, including serum creatinine, serum urea nitrogen, and 24-h urine output. Duration of AKI was defined as the number of days between the date of the serum creatinine increment and the study enrolment date. At enrolment, sepsis was ascertained using the systemic inflammatory response syndrome criteria, (Bone 1996) and two severity-of-illness scores were calculated, the Acute Physiology and Chronic Health Evaluation (APACHE) II score (Knaus et al. 1985) and the multiple organ failure (MOF) score (Knaus and Wagner 1989). Heart failure was defined as class III or IV of the New York Heart Association criteria (The Criteria Committee of the New York Heart Association 1994). Pre-existing chronic kidney disease (CKD) was defined on the basis of a baseline estimated GFR of less than 60 mL/min/1.73 m^2, as calculated by the 4-variable modification of diet in renal disease (MDRD) study equation (Levey et al. 2000).

Predictor and outcome variables

The main predictive variables of interest were urinary α-GST and π-GST. At enrolment, urine samples were obtained either through a fresh void or from a Foley catheter, via its sample access port. All samples were collected in sterile standard collection tubes containing no additives, and were kept on ice and processed within 30 min after collection. Samples were centrifuged at 3000 rpm for 10 min at 4°C to remove cellular elements and a protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany) was added to the transferred supernatant. 1-mL aliquots were stored at –80°C until assayed. The median time between sample storage and biomarker measurement was 4.9 years. Samples were thawed for 1 h prior to biomarker measurement. α-GST and π-GST were measured by sandwich ELISA (Argutus Medical Ltd., Dublin, Ireland). The average inter- and intra-assay coefficient of variation for α-GST was 9.1 and 8.0%, respectively, and for π-GST, 7.5 and 2.0%, respectively. All measurements were performed in duplicate. The α-GST and π-GST results were normalized to urinary creatinine, and expressed as ng/mg of creatinine.

The main outcome variables were dialysis requirement, and the composite of dialysis requirement or in-hospital death, which accounts for survival bias. The decision to initiate dialysis was made by the treating physician.

Statistical analysis

Continuous variables were described as mean (with standard deviation) or median (with 25th and 75th percentile) and categorical variables as percentage. Comparisons between groups were made by the ANOVA or the nonparametric Kruskal–Wallis test for continuous variables, and by χ^2 test for categorical variables. For each biomarker, study participants were grouped according to three categories: undetectable levels, and < vs. ≥median values, where the median was calculated within the detectable levels.

Receiver-operating characteristic (ROC) curves was generated to explore the diagnostic performance of urinary α-GST and π-GST for predicting the two outcomes of interest. The 95% confidence interval (CI) for each area under-the-ROC curve (AUC) was calculated using the asymptotic normal approximation. To explore the

performance of the biomarkers in different settings, *post hoc* stratified analyses were performed where AUCs were generated according to baseline eGFR (< vs. ≥60 mL/min/1.73 m^2), pre-existing heart failure, sepsis, APACHE II score (< vs. ≥median), need for mechanical ventilation, MOF score (0 vs. ≥1), AKI duration (≤ vs. >2 days), and AKI stage (1–3) according to the AKI network classification (Mehta et al. 2007). The nonparametric method of DeLong et al (DeLong et al. 1988) was used to test whether differences in the AUC for each biomarker between the various settings were statistically more or less discriminating.

Multivariable logistic regression analyses were also performed to examine the association of the urinary α-GST and π-GST categories with the two pre-specified outcomes, after adjustment for the APACHE II score. The results are displayed as odds ratios ((OR) using for reference group subjects with urinary α-GST or π-GST below the median) with 95% CI.

All statistical analyses were performed using the SAS software (version 9.1; SAS Institute, Cary, NC). Differences were considered statistically significant at a *p* value of less than 0.05.

Results

Characteristics of the cohort stratified by urinary α-GST and π-GST levels

Between November 2003 and July 2009, a total of 257 study participants were enrolled. The present study includes 245 participants who underwent biomarker measurements. Of note, for α-GST measurement, three subjects had insufficient urine. For the 245 participants, mean age was 66 years, 54% were men, and 91% were of white race. Mean APACHE II score was 20, 76% were in the intensive care unit, and 25% required assisted mechanical ventilation. At enrolment, mean serum creatinine was 3.6 mg/dL, serum urea nitrogen 63 mg/dL, and 24-h urine output 1.3 L/day. Ninety-six patients (39%) required dialysis and the observed in-hospital mortality was 22%. Forty-seven percent experienced the composite outcome of dialysis requirement or in-hospital death. Among the 96 patients who required dialysis, 32 received dialysis before study enrolment. In this subgroup of patients, the median period between initiation of dialysis and study enrolment (with urine collection) was 1 day (inter-quartile range 1, 2). The urinary biomarkers were measured at a median of 2 (inter-quartile range 1, 3) days from the onset of AKI. Urinary α-GST was undetectable in 102 (42%) subjects and π-GST in 42 (17%) subjects.

Tables 1 and 2 display the enrolment characteristics of the cohort according to the three urinary α-GST and π-GST categories. In brief, patients with undetectable α-GST levels had a higher mean APACHE II score, a higher MOF score, and a higher serum creatinine compared to those with detectable levels (Table 1). Conversely, there was a significant trend toward a higher mean MOF score, a higher serum creatinine, and a lower urine output among patients with the highest (≥median) π-GST levels compared to those with lower (<median) and undetectable levels (Table 2). In addition, there was a lower prevalence of diabetes mellitus and heart failure among patients with the highest (≥median) π-GST levels compared to the other two groups.

ROC analyses for prognostic performance of urinary α-GST and π-GST

The results of the ROC analyses are summarized in Table 3. In brief, urinary π-GST predicted dialysis requirement

Table 1. Enrolment characteristics of the AKI cohort according to urinary α-GST categories.

	Urinary α-GST categories			
	Undetectable (*n* = 102)	<Median (*n* = 70)	≥Median (*n* = 70)	
Variable	–	5.4 (2.9, 8.6) ng/mg	36.1 (19.3, 72.2) ng/mg	*p* value for trend
Age, years	67 ± 16	65 ± 13	66 ± 15	0.48
Men, %	52	64	46	0.56
White race, %	89	90	93	0.44
Intensive care unit setting, %	80	63	80	0.73
APACHE II score	21 ± 6	18 ± 5	19 ± 7	<0.001
MOF score	1.3 ± 0.9	0.9 ± 0.8	1.0 ± 1.0	0.01
Assisted mechanical ventilation, %	29	17	24	0.35
Sepsis, %	47	31	49	0.97
Chronic kidney disease, %	74	68	62	0.12
Heart failure, %	11	19	20	0.09
Liver disease, %	9	7	4	0.26
Diabetes mellitus, %	43	39	54	0.19
Serum creatinine, mg/dL	3.7 ± 1.8	3.6 ± 1.8	3.3 ± 1.7	0.03
Serum urea nitrogen, mg/dL	64 ± 33	65 ± 30	59 ± 28	0.38
Urine output, L/day	1.3 ± 1.0	1.3 ± 1.1	1.4 ± 1.2	0.98
Urinary π-GST, ng/mg	211 (38, 812)	100 (19, 572)	277 (49, 1210)	0.09

Continuous variables are presented as mean ± standard deviation or median (25th, 75th percentile), and categorical variables as percentage. The following variables had missing data: Chronic kidney disease (*n* = 5), and urine output (*n* = 4). The *p* values are based on the nonparametric Kruskal–Wallis test.

Table 2. Enrolment characteristics of the AKI cohort according to urinary π-GST categories.

	Urinary π-GST categories			
	Undetectable ($n=42$)	<Median ($n=102$)	≥Median($n=101$)	
Variable	–	82 (44, 166) ng/mg	882 (617, 1753) ng/mg	p value for trend
Age, years	66 ± 12	67 ± 16	65 ± 16	0.54
Men, %	55	61	46	0.13
White race, %	98	89	89	0.18
Intensive care unit setting, %	93	63	81	0.75
APACHE II score	20 ± 7	19 ± 6	20 ± 6	0.46
MOF score	0.9 ± 0.9	1.0 ± 0.9	1.2 ± 1.0	0.03
Assisted mechanical ventilation, %	38	18	26	0.36
Sepsis, %	45	35	52	0.20
Chronic kidney disease, %	79	66	67	0.29
Heart failure, %	31	14	11	0.007
Liver disease, %	2	8	9	0.22
Diabetes mellitus, %	67	45	37	0.002
Serum creatinine, mg/dL	3.1 ± 1.1	3.4 ± 1.4	4.0 ± 2.2	0.02
Serum urea nitrogen, mg/dL	64 ± 34	63 ± 30	62 ± 31	0.92
Urine output, L/day	1.7 ± 1.3	1.3 ± 1.0	1.1 ± 1.0	0.008
Urinary α-GST, ng/mg	7.2 (0, 16.1)	2.4 (0, 12.3)	3.5 (0, 29.2)	0.31

Continuous variables are presented as mean ± standard deviation or median (25th, 75th percentile), and categorical variables as percentage. The following variables had missing data: Chronic kidney disease ($n=5$), urine output ($n=4$), and urinary α-GST ($n=3$). The p values are based on the nonparametric Kruskal–Wallis test.

Table 3. Receiver-operating characteristic (ROC) curve analyses examining the performance of urinary α-GST and π-GST for prediction of adverse outcomes in the AKI cohort.

Prediction model	Dialysis requirement AUC (95% CI)	Dialysis requirement or in-hospital death AUC (95% CI)
Urinary α-GST	0.56 (0.50, 0.63)	0.56 (0.49, 0.63)
Urinary π-GST	0.59 (0.53, 0.66)	0.58 (0.52, 0.65)
APACHE II score	0.69 (0.63, 0.76)	0.76 (0.70, 0.82)
Urinary α-GST + APACHE II score	0.70 (0.63, 0.76)	0.77 (0.71, 0.83)
Urinary π-GST + APACHE II score	0.70 (0.63, 0.76)	0.76 (0.70, 0.82)
Urinary α-GST + π-GST	0.61 (0.54, 0.69)	0.61 (0.54, 0.68)
Urinary α-GST + π-GST + APACHE II score	0.70 (0.64, 0.77)	0.77 (0.71, 0.83)

CI denotes confidence interval; AUC denotes area under-the-ROC curve.

(AUC 0.59; 95% CI 0.53, 0.66; $p=0.007$), whereas α-GST, did not (AUC 0.56; 95% CI 0.50, 0.63; $p=0.07$). Similarly, π-GST performed better than α-GST for predicting the composite outcome with an AUC of 0.58 (95% CI 0.52, 0.65; $p=0.01$), as compared to an AUC of 0.56 (95% CI 0.49, 0.63; $p=0.09$), respectively. The addition of π-GST to the APACHE II score slightly increased the performance of the severity of illness score for prediction of dialysis requirement, with a small increment in the AUC from 0.69 (95% CI 0.63, 0.76; $p<0.001$) to 0.70 (95% CI 0.63, 0.76; $p<0.001$). The difference between these two AUCs however was not significant ($p=0.93$). Similarly, the addition of π-GST to the APACHE II score marginally changed the performance of the severity of illness score for prediction of the composite outcome, with a small increment in the AUC from 0.76 (95% CI 0.70, 0.82; $p<0.001$) to 0.77 (95% CI 0.71, 0.83; $p<0.001$). The difference between these two AUCs was also not significant ($p=0.19$).

Despite the overall marginal performance of the two urinary biomarkers in the entire population, we performed *post hoc* subgroup analyses to explore their performance in various clinical settings. As shown in Figure 1, the distal tubular marker π-GST consistently displayed better performance for the prediction of dialysis requirement in most subgroups, especially among patients with baseline eGFR <60 mL/min/1.73 m^2 (AUC 0.61; 95% CI 0.53, 0.69; $p=0.007$) and oliguria (AUC 0.72; 95% CI 0.58, 0.85; $p=0.002$). However, π-GST performed poorly in patients with heart failure (AUC 0.55; 95% CI 0.37, 0.73; $p=0.60$). By contrast, the proximal tubular marker α-GST displayed poor performance in patients with oliguria (AUC 0.53; 95% CI 0.37, 0.69; $p=0.73$), and good performance in patients with stage-1 (AUC 0.66; 95% CI 0.55, 0.76; $p=0.004$) and stage-2 AKI (AUC 0.80; 95% CI 0.60, 1.00; $p=0.004$), and appeared to perform better than π-GST in patients with heart failure, although not reaching statistical significance (AUC 0.65; 95% CI 0.47, 0.83; $p=0.09$).

As shown in Figure 2, π-GST performed well for prediction of dialysis requirement or in-hospital death, irrespective of baseline eGFR or duration of AKI at time of biomarker measurement. Although π-GST also displayed better performance in patients with oliguria (AUC 0.73;

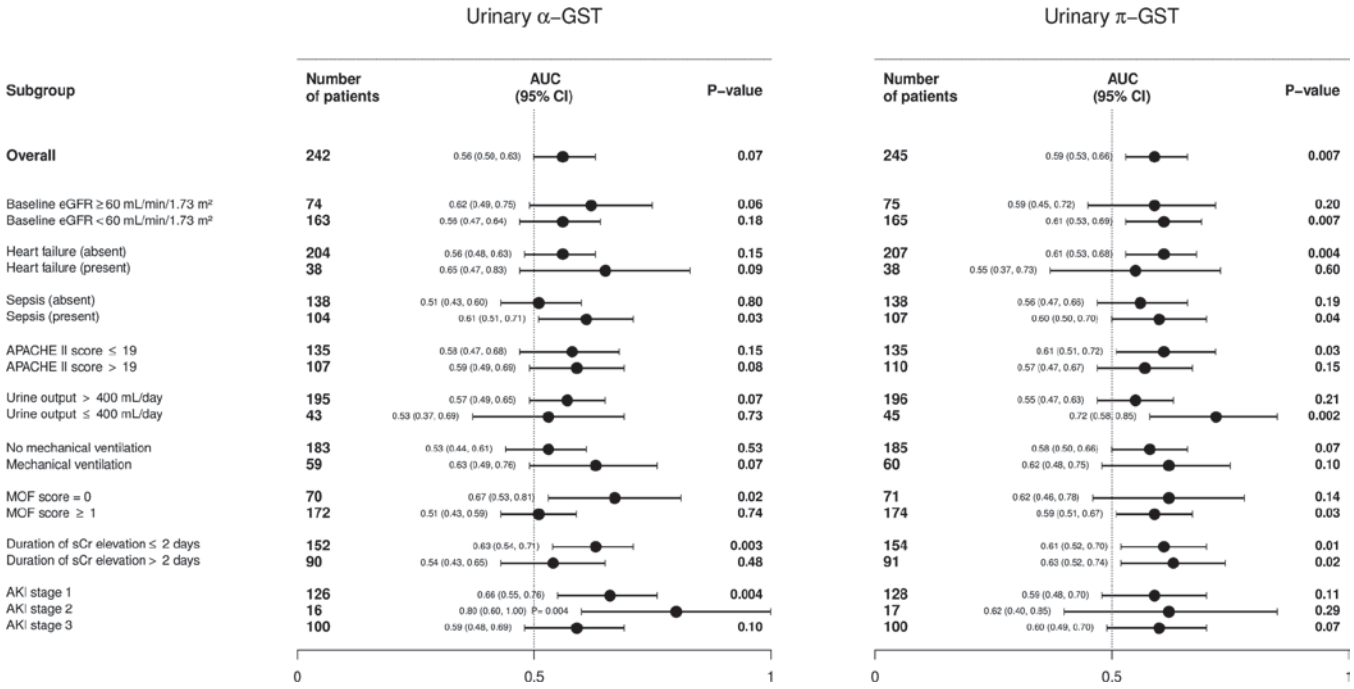

Figure 1. Receiver-operating characteristic (ROC) curve subgroup analyses examining the performance of urinary α-GST and π-GST for prediction of dialysis requirement. AUC denotes the area under-the-ROC curve.

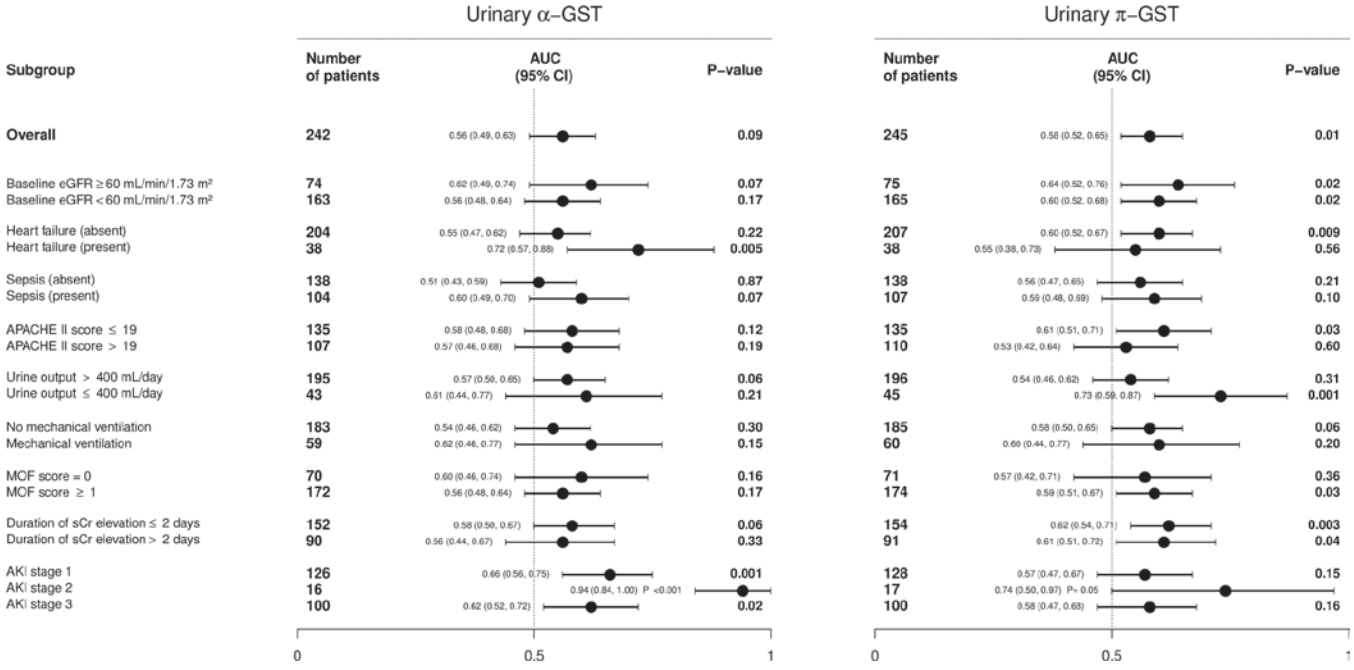

Figure 2. Receiver-operating characteristic (ROC) curve subgroup analyses examining the performance of urinary α-GST and π-GST for prediction of dialysis requirement or in-hospital death. AUC denotes the area under-the-ROC curve.

95% CI 0.60, 0.87; $p = 0.001$), this distal tubular marker performed poorly in patients with heart failure (AUC 0.55; 95% CI 0.38, 0.73; $p = 0.56$). A similar pattern was observed in which the proximal tubular marker α-GST did not perform well in oliguric patients, but displayed its best performance in patients with heart failure (AUC 0.72; 95% CI 0.57, 0.88; $p = 0.005$), and in all three stages of AKI.

In a sensitivity analysis that excluded the 32 patients who received dialysis before study enrolment, the performance of α-GST for prediction of dialysis requirement or in-hospital death strengthened (AUC 0.58; 95% CI 0.51, 0.65) whereas that of π-GST weakened (AUC 0.57; 95% CI 0.50, 0.65).

Urinary α-GST and π-GST and prediction of dialysis requirement

Next, we examined the association of the three urinary biomarker categories with dialysis requirement

Table 4. Logistic regression analyses examining the association of urinary α-GST and π-GST with adverse outcomes in the AKI cohort.

	Biomarker category			
	Undetectable	<Median (Reference)	≥Median	Wald χ^2 *p* value
Urinary α-GST				
Dialysis requirement				
Unadjusted OR (95% CI)	1.84 (0.97, 3.51)	1.0	1.56 (0.77, 3.13)	0.17
Adjusted OR (95% CI)	1.39 (0.71, 2.73)	1.0	1.54 (0.74, 3.19)	0.48
Dialysis requirement or in-hospital death				
Unadjusted OR (95% CI)	1.76 (0.95, 3.28)	1.0	1.79 (0.91, 3.52)	0.14
Adjusted OR (95% CI)	1.14 (0.57, 2.28)	1.0	1.98 (0.93, 4.21)	0.17
Urinary π-GST				
Dialysis requirement				
Unadjusted OR (95% CI)	1.80 (0.85, 3.79)	1.0	2.17 (1.22, 3.87)*	0.03
Adjusted OR (95% CI)	1.74 (0.79, 3.82)	1.0	2.12 (1.16, 3.90)**	0.05
Dialysis requirement or in-hospital death				
Unadjusted OR (95% CI)	1.62 (0.78, 3.33)	1.0	2.01 (1.15, 3.52)**	0.05
Adjusted OR (95% CI)	1.58 (0.70, 3.56)	1.0	1.99 (1.07, 3.72)†	0.09

The analyses are adjusted for the APACHE II score.
CI, confidence interval; OR, odds ratio.
*$p = 0.01$, **$p = 0.02$, and †$p = 0.03$ for comparisons between ≥median vs. <median biomarker category.

(Table 4). In the unadjusted analysis, compared with the lower (<median) category, the higher (≥median) urinary π-GST category was associated with an OR of 2.17 for dialysis requirement (95% CI 1.22, 3.87; $p = 0.01$), which persisted after adjustment for the APACHE II score (OR 2.12; 95% CI 1.16, 3.90; $p = 0.02$), as well as in a model that included the APACHE II score, sepsis, assisted mechanical ventilation, and liver disease (OR 2.15; 95% CI 1.16, 3.98; $p = 0.02$).

Compared with the lower (<median) category, there was no association between the higher (≥median) urinary α-GST category and dialysis requirement. However, there was a nonsignificant association between the undetectable urinary α-GST category (vs. <median) and dialysis requirement (OR 1.84; 95% CI 0.97, 3.51; $p = 0.06$), which was further attenuated after adjustment for the APACHE II score (OR 1.39; 95% CI 0.71, 2.73; $p = 0.34$). Of note, the duration of AKI was not independently associated with dialysis requirement (data not shown).

Urinary α-GST and π-GST and prediction of dialysis requirement or in-hospital death

Finally, we examined the association of the urinary biomarker categories with the composite of dialysis requirement or in-hospital death (Table 4). In the unadjusted analysis, compared with the lower (<median) category, the higher (≥median) urinary π-GST category was associated with an OR of 2.01 for the composite outcome (95% CI 1.15, 3.52; $p = 0.02$), which persisted after adjustment for the APACHE II score (OR 1.99; 95% CI 1.07, 3.72; $p = 0.03$), as well as in a model that included the APACHE II score, sepsis, mechanical ventilation, and liver disease (OR 1.94; 95% CI 1.03, 3.65; $p = 0.04$).

There was a nonsignificant association between the higher (≥median) urinary α-GST category (vs. <median) and the composite outcome (OR 1.79; 95% CI 0.91, 3.52; $p = 0.09$), which was attenuated after adjustment for the APACHE II score (OR 1.98; 95% CI 0.93, 4.21; $p = 0.08$). Similarly, there was a nonsignificant association between the undetectable urinary α-GST category (vs. <median) and the composite outcome (OR 1.76; 95% CI 0.95, 3.28; $p = 0.07$), which was further attenuated after adjustment for the APACHE II score (OR 1.14; 95% CI 0.57, 2.28; $p = 0.71$). Similarly, the duration of AKI was not independently associated with the composite outcome (data not shown).

Discussion

There is an ongoing search for markers that improve the ability to predict recovery or evolution of hospital-acquired AKI, including dialysis requirement, as this might potentially facilitate timely treatment initiation. In the present study, we evaluated the prognostic utility of two urinary leakage markers, α-GST and π-GST, measured at the time of nephrology consultation, at a median of 2.0 days following the diagnosis of AKI, for predicting dialysis requirement. Since α-GST and π-GST have respective specificity for the proximal and distal tubule (Feinfeld et al. 1977, Harrison et al. 1989, Campbell et al. 1991), we hypothesized that these two markers would provide additive value in predicting severity of nephron injury that would result in dialysis requirement. To overcome the potential for survival bias, we also explored the composite endpoint of dialysis requirement or in-hospital death. Urinary π-GST performed better than α-GST for predicting dialysis requirement (AUC of 0.59) and the composite endpoint (AUC of 0.58), although its performance was modest at best. The addition of either urinary marker to the APACHE II score did not improve model discrimination to predict dialysis requirement

or the composite outcome. On subgroup exploratory analyses, the distal tubular marker π-GST consistently displayed better performance for the prediction of dialysis requirement among patients with pre-existing CKD (AUC of 0.61), and those with oliguria (AUC of 0.72). However, these subgroup analyses must be interpreted with caution, and require further confirmation. When stratified by urinary biomarker level categories, compared with the lower (<median) category, the higher (≥median) urinary π-GST category was associated with 2-fold increased adjusted odds for dialysis requirement or the composite endpoint.

To our knowledge, this is the largest prospective cohort study testing the hypothesis of urinary α-GST and π-GST predicting clinical end-points in AKI with a focus on dialysis requirement. Several previous studies have evaluated the role of α-GST and π-GST for early detection of AKI (Eger et al. 1997, Boldt et al. 2003, Westhuyzen et al. 2003, da Silva Magro and de Fatima Fernandes Vattimo 2004, Eijkenboom et al. 2005, Yavuz et al. 2009, Koyner et al. 2010, Walshe et al. 2009). However, only two studies have examined the role of these markers for prediction of adverse outcomes in patients with established AKI. (Koyner et al. 2010, Herget-Rosenthal et al. 2004) Herget-Rosenthal et al found urinary α-GST to predict dialysis requirement in 73 patients with non-oliguric acute tubular necrosis (AUC of 0.64) (Herget-Rosenthal et al. 2004). Similarly, Koyner et al found excellent performance of urinary π-GST (AUC of 0.84) for predicting evolution of stage-1 to stage-3 AKI in a subgroup of 46 patients following cardiac surgery, but not of α-GST (AUC of 0.54) (Koyner et al. 2010). Other studies have also evaluated these two markers in the differential diagnosis of kidney allograft dysfunction, whereby an isolated increase in π-GST was associated with acute cellular rejection as opposed to other causes (Sundberg et al. 1994b, Kuzniar et al. 2006). In addition, π-GST has been shown to be elevated in septic patients irrespective of AKI (Walshe et al. 2009).

Strengths of our study include the relatively larger sample size compared to the prior reports (Herget-Rosenthal et al. 2004, Koyner et al. 2010), and a fairly consistent performance of π-GST across several subgroup analyses. The main study limitation is the measurement of the urinary biomarker at a single time-point, which introduces potential time bias. As mentioned above, in the study by Koyner et al (Koyner et al. 2010), among patients undergoing cardiac surgery, π-GST performed best at predicting outcomes in early stages of AKI. We can only speculate as to whether the performance of these urinary markers in our study was compromised due in part to the enrolment of patients with various stages of AKI. Indeed, in a sensitivity analysis where we excluded patients who received dialysis before study enrolment, the performance of α-GST improved, which is a clear indication of the heterogeneous study population. The incorporation of several measurements might have enhanced the predictive power of the two biomarkers, as their urinary appearance is likely to be time sensitive. We partially addressed this issue by adjusting the analyses for duration of AKI, which did not affect the overall results. Urinary α-GST and π-GST levels were undetectable in 42 and 17% of subjects, respectively. We addressed this finding analytically by stratifying each biomarker according to three categories for predicting the two outcomes of interest. We acknowledge that there are non-AKI related factors that might influence urinary α-GST and π-GST levels including, host and environmental factors (Aliya et al. 2003), sepsis (Walshe et al. 2009), and proteinuric kidney disease (Branten et al. 2000) to name a few. Our study does not allow us to fully explore the non-AKI determinants of these tubular leakage markers. Another limitation of the study is the use of the Hou criteria (Hou et al. 1983) to define AKI, which precedes the most recently accepted AKI network staging system. In a sensitivity analysis however, after reclassifying AKI according to this staging system, there was better discrimination of α-GST in earlier stages of AKI. The median time period between urine collection and GST measurement was 4.9 years. We are unaware of any published studies on the potential influence of prolonged urine sample storage on GST measurement. We feel confident, however, that by storing the urine samples at −80°C, we minimized risk of decay. In addition, all samples were treated with a protease inhibitor cocktail aimed at preventing protein degradation. Finally, the lack of detectable α-GST in 40% of the samples might be due in part to the heterogeneous nature and severity of AKI in our cohort resulting in variable α-GST urinary leakage. Of note, the α-GST assay employed an anti-α-GST polyclonal IgG antibody compared with the π-GST that employed an anti-π-GST monoclonal IgG antibody. The development of newer α-GST assays that utilize monoclonal antibodies might mitigate this potential problem.

In conclusion, in this hospital-based cohort of patients with AKI, a single urinary measurement of the distal tubular leakage marker π-GST obtained at the time of nephrology consultation provided modest prognostic discrimination for predicting dialysis requirement, whereas the proximal tubular marker α-GST did not. The potential prognostic value of these markers needs to be validated in larger cohorts and across a broader spectrum of clinical settings.

Declaration of interest

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References

Aliya S, Reddanna P, Thyagaraju K. (2003). Does glutathione S-transferase π (GST-π) a marker protein for cancer? *Mol Cell Biochem* 253:319–327.

Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P; Acute Dialysis Quality Initiative workgroup. (2004). Acute renal failure – definition, outcome measures, animal models, fluid therapy and information technology needs: The Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 8:R204–R212.

Boldt J, Brenner T, Lang J, Kumle B, Isgro F. (2003). Kidney-specific proteins in elderly patients undergoing cardiac surgery with cardiopulmonary bypass. *Anesth Analg* 97:1582–1589.

Bone RC. (1996). Sir Isaac Newton, sepsis, SIRS, and CARS. *Crit Care Med* 24:1125–1128.

Bonventre JV, Weinberg JM. (2003). Recent advances in the pathophysiology of ischemic acute renal failure. *J Am Soc Nephrol* 14:2199–2210.

Branten AJ, Mulder TP, Peters WH, Assmann KJ, Wetzels JF. (2000). Urinary excretion of glutathione S transferases α and π in patients with proteinuria: Reflection of the site of tubular injury. *Nephron* 85:120–126.

Campbell JA, Corrigall AV, Guy A, Kirsch RE. (1991). Immunohistologic localization of α, μ, and π class glutathione S-transferases in human tissues. *Cancer* 67:1608–1613.

Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. (2005). Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 16:3365–3370.

Coca SG, Yalavarthy R, Concato J, Parikh CR. (2008). Biomarkers for the diagnosis and risk stratification of acute kidney injury: A systematic review. *Kidney Int* 73:1008–1016.

da Silva Magro MC, de Fatima Fernandes Vattimo M. (2004). Does urinalysis predict acute renal failure after heart surgery? *Ren Fail* 26:385–392.

DeLong ER, DeLong DM, Clarke-Pearson DL. (1988). Comparing the areas under two or more correlated receiver operating characteristic curves: A nonparametric approach. *Biometrics* 44:837–845.

Dieterle F, Sistare F. (2010). Biomarkers of acute kidney injury. In: Vaidya V, Bonventre J. (eds). *Biomarkers in Medicine, Drug Discovery, and Environmental Health*. New York: John Wiley and Sons, Inc.

Eger EI 2nd, Koblin DD, Bowland T, Ionescu P, Laster MJ, Fang Z, Gong D, Sonner J, Weiskopf RB. (1997). Nephrotoxicity of sevoflurane versus desflurane anesthesia in volunteers. *Anesth Analg* 84:160–168.

Eijkenboom JJ, van Eijk LT, Pickkers P, Peters WH, Wetzels JF, van der Hoeven HG. (2005). Small increases in the urinary excretion of glutathione S-transferase A1 and P1 after cardiac surgery are not associated with clinically relevant renal injury. *Intensive Care Med* 31:664–667.

Feinfeld DA, Bourgoignie JJ, Fleischner G, Goldstein EJ, Biempica L, Arias IM. (1977). Ligandinuria in nephrotoxic acute tubular necrosis. *Kidney Int* 12:387–392.

Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A; NGAL Meta-analysis Investigator Group. (2009). Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: A systematic review and meta-analysis. *Am J Kidney Dis* 54:1012–1024.

Harrison DJ, Kharbanda R, Cunningham DS, McLellan LI, Hayes JD. (1989). Distribution of glutathione S-transferase isoenzymes in human kidney: Basis for possible markers of renal injury. *J Clin Pathol* 42:624–628.

Herget-Rosenthal S, Poppen D, Hüsing J, Marggraf G, Pietruck F, Jakob HG, Philipp T, Kribben A. (2004). Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. *Clin Chem* 50:552–558.

Hou SH, Bushinsky DA, Wish JB, Cohen JJ, Harrington JT. (1983). Hospital-acquired renal insufficiency: A prospective study. *Am J Med* 74:243–248.

Knaus WA, Draper EA, Wagner DP, Zimmerman JE. (1985). APACHE II: A severity of disease classification system. *Crit Care Med* 13:818–829.

Knaus WA, Wagner DP. (1989). Multiple systems organ failure: Epidemiology and prognosis. *Crit Care Clin* 5:221–232.

Koyner JL, Vaidya VS, Bennett MR, Ma Q, Worcester E, Akhter SA, Raman J, Jeevanandam V, O'Connor MF, Devarajan P, Bonventre JV, Murray PT. (2010). Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. *Clin J Am Soc Nephrol* 5:2154–2165.

Kuzniar J, Marchewka Z, Krasnowski R, Boratynska M, Dlugosz A, Klinger M. (2006). Enzymuria and low molecular weight protein excretion as the differentiating marker of complications in the early post kidney transplantation period. *Int Urol Nephrol* 38: 753–758.

Levey AS, Greene T, Kusek JW, Beck GJ. A simplified equation to predict glomerular filtration rate from serum creatinine. *J Am Soc Nephrol* 2000;11:A0828. (abstr)

Levy EM, Viscoli CM, Horwitz RI. (1996). The effect of acute renal failure on mortality. A cohort analysis. *JAMA* 275:1489–1494.

Liangos O, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H, MacKinnon RW, Li L, Balakrishnan VS, Pereira BJ, Bonventre JV, Jaber BL. (2007). Urinary N-acetyl-β-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. *J Am Soc Nephrol* 18:904–912.

Liangos O, Wald R, O'Bell JW, Price L, Pereira BJ, Jaber BL. (2006). Epidemiology and outcomes of acute renal failure in hospitalized patients: A national survey. *Clin J Am Soc Nephrol* 1:43–51.

Mannervik B. (1985). The isoenzymes of glutathione transferase. *Adv Enzymol Relat Areas Mol Biol* 57:357–417.

McMahon BA, Koyner JL, Murray PT. (2010). Urinary glutathione s-transferases in the pathogenesis and diagnostic evaluation of acute kidney injury following cardiac surgery: A critical review. *Curr Opin Crit Care* 16:550–555.

Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A; Acute Kidney Injury Network. (2007). Acute Kidney Injury Network: Report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 11:R31.

Moran SM, Myers BD. (1985). Course of acute renal failure studied by a model of creatinine kinetics. *Kidney Int* 27:928–937.

Nash K, Hafeez A, Hou S. (2002). Hospital-acquired renal insufficiency. *Am J Kidney Dis* 39:930–936.

Perianayagam MC, Seabra VF, Tighiouart H, Liangos O, Jaber BL. (2009). Serum cystatin C for prediction of dialysis requirement or death in acute kidney injury: A comparative study. *Am J Kidney Dis* 54:1025–1033.

Rosner MH, Okusa MD. (2006). Acute kidney injury associated with cardiac surgery. *Clin J Am Soc Nephrol* 1:19–32.

Star RA. (1998). Treatment of acute renal failure. *Kidney Int* 54:1817–1831.

Sundberg A, Appelkvist EL, Dallner G, Nilsson R. (1994a). Glutathione transferases in the urine: Sensitive methods for detection of kidney damage induced by nephrotoxic agents in humans. *Environ Health Perspect* 102 suppl 3:293–296.

Sundberg AG, Appelkvist EL, Bäckman L, Dallner G. (1994). Urinary pi-class glutathione transferase as an indicator of tubular damage in the human kidney. *Nephron* 67:308–316.

The Criteria Committee of the New York Heart Association. (1994). *Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels*, Boston, Little, Brown and Company.

Walshe CM, Odejayi F, Ng S, Marsh B. (2009). Urinary glutathione S-transferase as an early marker for renal dysfunction in patients admitted to intensive care with sepsis. *Crit Care Resusc* 11:204–209.

Westhuyzen J, Endre ZH, Reece G, Reith DM, Saltissi D, Morgan TJ. (2003). Measurement of tubular enzymuria facilitates early detection of acute renal impairment in the intensive care unit. *Nephrol Dial Transplant* 18:543–551.

Yavuz I, Asgun FH, Bolcal C, Bingol H, Yokusoglu M, Baysan O, Ozgurtas T, Demirkilic U, Tatar H. (2009). Importance of urinary measurement of glutathione S-transferase in renal dysfunction patients after on- and off-pump coronary artery bypass surgery. *Thorac Cardiovasc Surg* 57:125–129.