

# Urinary glutathione S-transferase as an early marker for renal dysfunction in patients admitted to intensive care with sepsis

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Acute kidney injury (AKI) is a common organ dysfunction, identifiable in about 50% of patients admitted to intensive care units with the diagnosis of sepsis.<sup>1</sup> In 2008, 51% of patients admitted with sepsis to ICUs in Ireland developed AKI.<sup>2</sup> Hospitalised patients who develop AKI continue to have a fivefold higher mortality than those with normal renal function.<sup>3</sup> The requirement for renal replacement therapy (RRT) in ICU patients with AKI has been associated with an excess risk of in-hospital death.<sup>1,4</sup>

Diagnosis of AKI currently relies on measurement of serum creatinine level and urine flow, which are components of both the RIFLE (Risk, Injury, Failure, Loss, End Stage Kidney Disease<sup>5</sup>) and AKIN (Acute Kidney Injury Network<sup>6</sup>) classification systems. These parameters have the disadvantages of low specificity and sensitivity, and slow rate of change, thus limiting early diagnosis of AKI. Earlier detection of AKI could allow early aggressive renal salvage therapies to be instituted, which, together with the associated improved monitoring of response to therapy, could improve renal function, allow RRT to be avoided, and decrease morbidity and mortality.

A range of biomarkers for AKI that potentially reflect different patterns of subcellular injury have been studied, including neutrophil gelatinase-associated lipocalin (NGAL).<sup>7</sup> Haase-Fielitz et al<sup>8</sup> examined the usefulness of serum biomarkers in predicting AKI in patients on arrival in the ICU after cardiac surgery. They found that the novel biomarkers serum NGAL and serum cystatin C were good predictors of AKI, while the conventional markers — serum urea and creatinine were poor predictors.

Three main sources of urinary biomarkers of AKI have been identified: lysosomes, brush-border membrane and cell cytoplasm.<sup>9</sup> The glutathione S-transferase (GST) protein family protects cells by inactivating reactive compounds through the action of reduced glutathione before their excretion into urine or bile. Specific isoforms of GST are found in the cytoplasm of cells in different regions of the nephron:  $\alpha$ -GST (molecular weight, 52 kDa) and  $\pi$ -GST (molecular weight, 49.4 kDa) are found in cells lining the lumen of the proximal and distal tubule, respectively.<sup>10</sup> These two isoforms “leak” into the

## ABSTRACT

**Objective:** Diagnosis of acute kidney injury (AKI) relies on measurement of serum creatinine concentration and urine flow, which change slowly and have low specificity and sensitivity. We investigated the potential of urinary levels of  $\alpha$ -glutathione S-transferase ( $\alpha$ -GST) and  $\pi$ -GST — markers of proximal and distal renal tubule damage, respectively — to provide an earlier and more accurate indication of AKI in patients in the intensive care unit.

**Design, setting and participants:** Urine samples were collected over the 48 hours after ICU admission from 40 consecutive patients who were admitted with a diagnosis of sepsis between October 2007 and May 2008. AKI was diagnosed during the 48h after ICU admission with the criteria of the Acute Kidney Injury Network (AKIN). Urinary  $\alpha$ -GST and  $\pi$ -GST levels were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits. Serum creatinine concentration was also measured. Haemodynamic and resuscitation parameters were recorded, but managed independently by the ICU team.

**Results:** Urine samples were analysed from 38 patients (21 men, 17 women) with a median age of 54 years (interquartile range [IQR], 41–69 years), median APACHE II score of 13.3 (IQR, 8–17), and median ICU length of stay of 9 days (IQR, 3–19 days). Hospital mortality was 24%, and ICU mortality was 13%. Nineteen patients (50%) developed AKI, all within 24h of ICU admission. Urinary  $\alpha$ -GST level was not increased in patients who developed AKI versus non-AKI patients. Median (IQR) urinary  $\pi$ -GST level ( $\mu\text{g/L}$ ) at ICU admission was 10.8 (4.7–22.65) in the non-AKI group, 19.3 (2.88–44) in those who developed Stage 1 AKI, and 27.4 (14.8–43.8) in those who developed Stage 3 AKI. Median urinary  $\pi$ -GST level at ICU admission was higher in all groups than in healthy control subjects. The area under the receiver operating characteristics curve for urinary  $\pi$ -GST level indicated that it was not a good predictor of AKI.

**Conclusions:** Urinary  $\pi$ -GST is elevated early in all patients with sepsis syndrome, but is not predictive of AKI as defined by AKIN. This may indicate sensitive detection of an earlier phase of kidney injury, and suggests that sepsis-related renal injury affects the distal tubules, giving new insights into the pathophysiology of AKI in sepsis.

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**Table 1. Diagnostic and staging criteria for acute kidney injury<sup>6</sup>**

Stage	Serum creatinine criteria	Urine output criteria
1	Increase to $\geq 26.4 \mu\text{mol/L}$ or increase $\geq 1.5$ to 2-fold from baseline	$< 0.5 \text{ mL/kg}$ per hour for more than 6 h
2	Increase $> 2$ to 3-fold from baseline	$< 0.5 \text{ mL/kg}$ per hour for more than 12 h
3*	Increase $> 3$ -fold from baseline or serum creatinine $\geq 354 \mu\text{mol/L}$ with an acute increase of at least $44 \mu\text{mol/L}$	$< 0.3 \text{ mL/kg}$ per hour for 24 h or anuria for 12 h

\* All patients who receive renal replacement therapy are considered to meet Stage 3 criteria.

**Table 2. Urinary glutathione S-transferase levels ( $\mu\text{g/L}$ ) in 100 healthy volunteers with normal kidney function**

	Range	Median	Interquartile range
$\alpha$ -GST	0–15	2.4	1.2–5.5
$\pi$ -GST	0–32	1.5	0.45–6.75

GST = glutathione S-transferase.

urine when cells are damaged. In contrast, GST from other tissues (eg, liver, small intestine, testis, ovaries and adrenal glands) does not cross the glomerulus because of its high molecular weight. Elevated GST levels in urine thus indicate damage specific to the source cells.

Previous prospective studies of  $\alpha$ -GST and  $\pi$ -GST in critically ill patients showed a high degree of sensitivity for predicting AKI and RRT requirement.<sup>11,12</sup> However, few studies included patients with sepsis, and significant heterogeneity was evident in patient populations, severity of illness and presence of confounding factors.

We hypothesised that  $\alpha$ -GST and  $\pi$ -GST are detectable in urine earlier in the course of kidney injury than existing

indicators of renal damage or insult (such as a rise in serum creatinine concentration), and may have potential as early markers of renal injury. To examine this hypothesis we measured urinary  $\alpha$ -GST and  $\pi$ -GST levels in patients with sepsis syndrome. Secondly, we studied the time course of urinary GST release versus serum creatinine changes. Finally, we considered whether these biomarkers could be clinically useful.

## Methods

Following approval from the Research Ethics Committee of the Mater Misericordiae University Hospital, Dublin, Ireland, we studied 40 consecutive patients admitted to the ICU with a diagnosis of sepsis between October 2007 and May 2008. As the study involved urine sampling, preservation of urine flow was required. Sepsis was diagnosed with the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference:<sup>13</sup>

- a presumed or confirmed focus of infection; and
- two or more of:
  - core temperature  $> 38^\circ \text{C}$  or  $< 36^\circ \text{C}$ ;
  - white cell count  $> 12 \times 10^9/\text{L}$  or  $< 4 \times 10^9/\text{L}$ , or  $> 10\%$  immature neutrophils;
  - tachycardia (heart rate  $> 90$  beats per min); or
  - tachypnoea (respiratory rate  $> 20$  breaths per min) or  $\text{Paco}_2 < 32 \text{ mmHg}$  or mechanical ventilation.

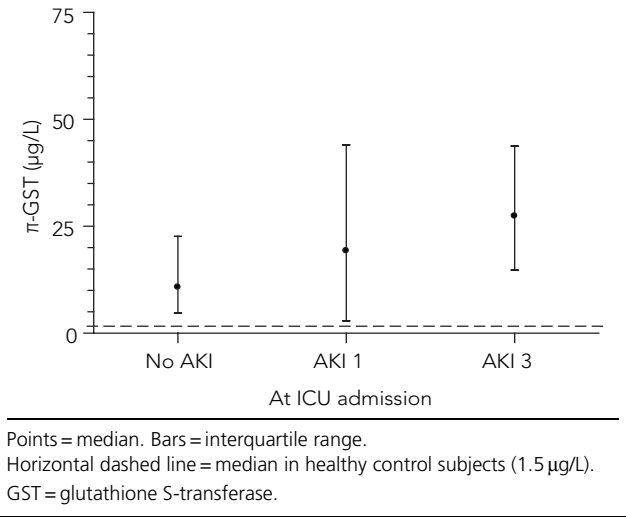
Urine samples were collected from recruited patients on ICU admission and then 4-hourly over the ensuing 48 hours. Samples were stored at  $-20^\circ \text{C}$  before analysis. Urinary  $\alpha$ -GST and  $\pi$ -GST levels were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits (Nephkit Alpha GST EIA and PI GST EIA, Argutus Medical, Dublin, Ireland). Serum creatinine level was measured at 0, 24 and 48 hours after ICU admission. Intensive care clinical management was not standardised and was as directed by the intensive care team. The ICU teams were blinded to the GST results.

**Table 3. Patient characteristics, by acute kidney injury (AKI) status**

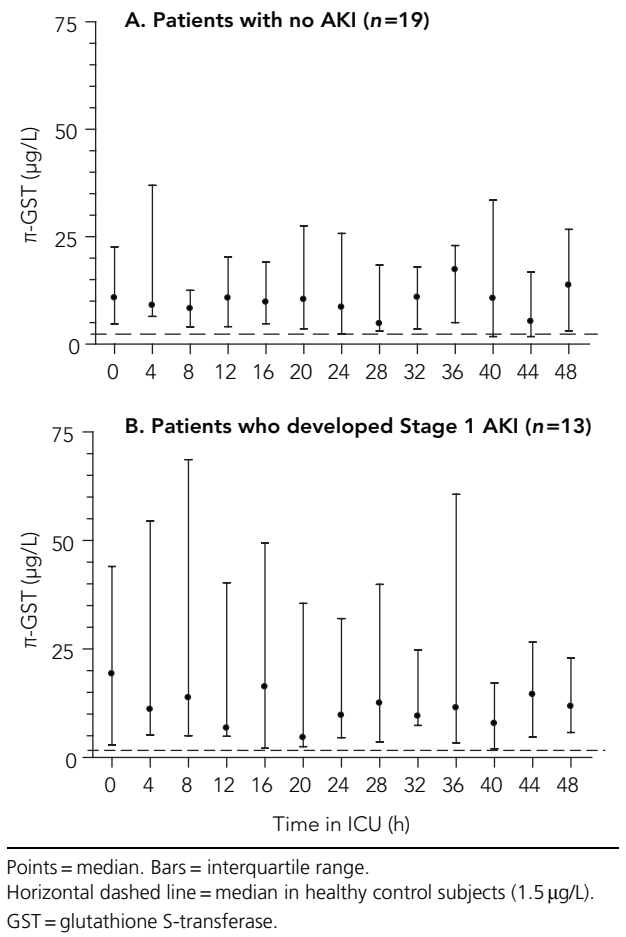
	All patients ( <i>n</i> = 38)	No AKI ( <i>n</i> = 19)	Stage 1 AKI ( <i>n</i> = 13)	Stage 2 AKI ( <i>n</i> = 2)	Stage 3 AKI ( <i>n</i> = 4)
Age (years), median (IQR)	54 (41–69)	55 (37–69)	45 (38–72)	58.5 (50–67)	60 (55–67)
APACHE score, median (IQR)	13.3 (8–17)	14 (5–16)	12 (9–18)	9.5 (6–13)	21 (16–22)
ICU LOS (days), median (IQR)	9 (3–19)	12 (3–20)	10 (3.5–19)	2.5 (1–4)	13 (5.5–56)
ICU mortality, no. (%)	5 (13%)	4 (21%)	1 (8%)	0	0
Hospital mortality, no. (%)	9 (24%)	4 (21%)	3 (23%)	0	1 (25%)
Inotropic support, no. (%)	27 (71%)	12 (63%)	10 (77%)	1 (50%)	4 (100%)

IQR = interquartile range. LOS = length of stay.

**Figure 1. Urinary  $\pi$ -GST concentration at ICU admission, by acute kidney injury (AKI) status**



**Figure 2. Urinary  $\pi$ -GST concentration over time after ICU admission, by acute kidney injury (AKI) status**



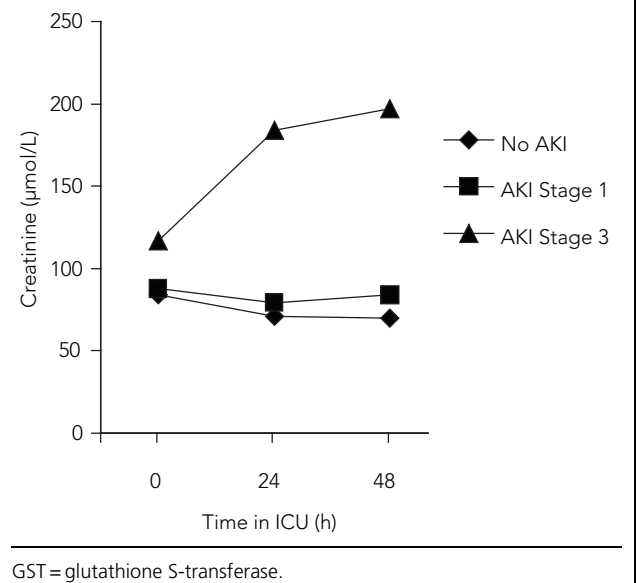
AKI was diagnosed and classified according to the criteria of AKIN<sup>6</sup> (Table 1). Urinary levels of  $\alpha$ -GST and  $\pi$ -GST were compared between ICU patients who developed AKI, those who did not develop AKI, and healthy volunteers (controls); data for the last group were supplied by Argutus Medical (Table 2).

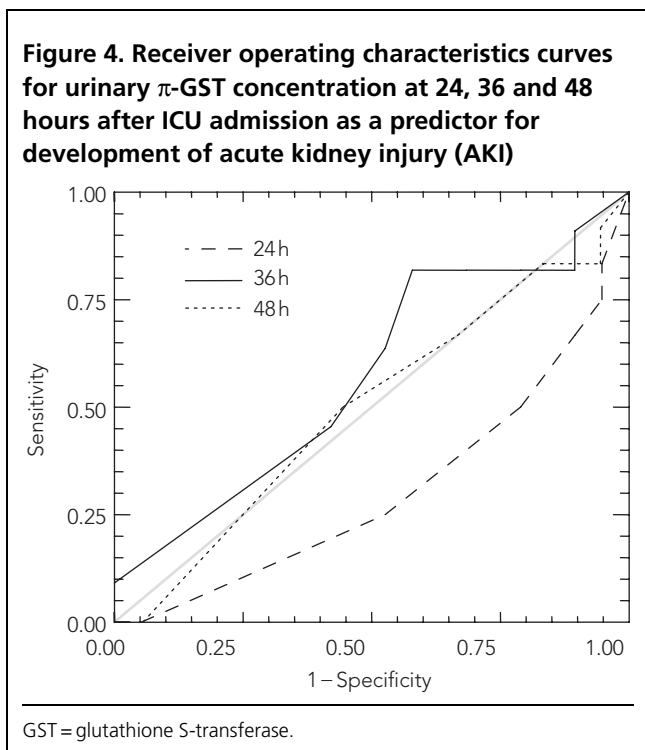
Data are presented as proportions for categorical variables, mean and standard deviation for normally distributed continuous variables, and median and interquartile range (IQR) for non-normally distributed continuous variables. Statistical analysis was performed using the Kolmogorov–Smirnov test for normality of variables and Wilcoxon signed-rank test for comparison of medians (GraphPad Prism 4, GraphPad Software, La Jolla, Calif, USA). The area under the receiver operating characteristics (ROC) curve for  $\pi$ -GST as a predictor of AKI was calculated.

**Results**

Urine samples were collected from 40 patients. Two patients died before the second sample was collected and were excluded from the analysis. The remaining 38 comprised 21 men and 17 women. Their demographic and clinical characteristics are shown in Table 3. AKI was diagnosed in 19 patients (50%): Stage 1 in 13 (34% of all patients), Stage 2 in two (5%), and Stage 3 in four (11%). All who developed AKI did so within 24 hours of ICU admission.

**Figure 3. Median serum creatinine concentration, by acute kidney injury (AKI) status**





Of the 13 patients diagnosed with Stage 1 AKI, seven met the criteria for a rise in serum creatinine concentration (increase to 26.4  $\mu\text{mol/L}$  or more, or a 1.5 to 2-fold increase from baseline), and six met the criterion for oliguria ( $<0.5$  mL/kg per hour for more than 6 hours). As only two patients met criteria for Stage 2 AKI, statistical analysis was not possible in this group. In all patients with Stage 3 AKI, diagnosis was based on the requirement for RRT.

Urinary  $\alpha$ -GST level was overall no higher in patients who developed AKI than in those who did not: median (IQR) on admission was 0.89  $\mu\text{g/L}$  (0.1–10.7  $\mu\text{g/L}$ ) in those who developed AKI versus 3.4  $\mu\text{g/L}$  (0.34–11.9  $\mu\text{g/L}$ ) in those who did not develop AKI ( $P=0.34$ ).

Urinary  $\pi$ -GST levels at ICU admission are shown in Figure 1 by AKI group. Median (IQR) values were 10.8  $\mu\text{g/L}$  (4.7–22.65  $\mu\text{g/L}$ ) for patients who did not develop AKI, 19.3  $\mu\text{g/L}$  (2.88–44  $\mu\text{g/L}$ ) for those who developed Stage 1 AKI, and 27.4  $\mu\text{g/L}$  (14.8–43.8  $\mu\text{g/L}$ ) for those who developed Stage 3 AKI. The difference between groups for median  $\pi$ -GST level did not reach statistical significance ( $P=0.45$ ).

Figure 2 shows urinary  $\pi$ -GST levels over time in patients who developed Stage 1 AKI compared with patients who did not develop AKI and the healthy control group (who had a median  $\pi$ -GST level of 1.5  $\mu\text{g/L}$ ). For all time intervals, the median urinary  $\pi$ -GST level was statistically greater in patients who did not develop AKI and in those with Stage 1 AKI than in the control group ( $P<0.001$  and  $P<0.005$ , respectively). It was also greater in patients with Stage 3 AKI

than in the control group, but the difference was not statistically significant ( $P=0.13$ ) (not shown). Figure 3 shows serum creatinine concentration over time, by AKI status.

The ROC curve for urinary  $\pi$ -GST level as a predictor of AKI is shown in Figure 4; calculation of the area under the curve indicated that  $\pi$ -GST level was not a good predictor of AKI.

## Discussion

AKI remains a significant problem for ICU patients with sepsis. It continues to have a high mortality, with a hospital mortality of 60.3% found in patients with AKI studied by the BEST (Beginning and Ending Supportive Therapy for the Kidney) investigators in 2005.<sup>1</sup> Sepsis was the predominant cause of AKI in 47.5% of these patients.

It is well documented that serum creatinine concentration is an insensitive marker of renal dysfunction, as it is affected by many other factors, including muscle mass and injury, some drugs, diet, ketosis and haemolysis. Similarly, urea concentration is affected by diet, gastrointestinal haemorrhage, liver disease, trauma and sepsis.<sup>14</sup> These biochemical indices must clearly be interpreted in the context of all these variables. There is therefore a need for more sensitive and specific measures of renal injury, to enable earlier diagnosis, identification of injurious therapies, and evaluation of therapeutic response.

Our objective in this prospective study was to evaluate the potential of the urinary biomarkers  $\alpha$ -GST and  $\pi$ -GST to provide early accurate diagnosis of AKI in a cohort of 40 consecutive patients admitted to the ICU with a diagnosis of sepsis syndrome. Although previous prospective studies of  $\alpha$ -GST and  $\pi$ -GST found these markers to have high sensitivity for predicting AKI and requirement for RRT,<sup>11,12</sup> the patients included had significant heterogeneity, and few had sepsis. In addition, the study of Westhuyzen et al used patients who did not develop AKI as the control group for urinary  $\pi$ -GST.<sup>11</sup> As the patient cohort comprised 26 consecutive emergency admissions to the ICU, all were critically ill and unlikely to have “normal” renal function whether or not they met the biochemical diagnosis of AKI. Of note, 23% of the patient cohort was receiving vasopressor therapy, which may have masked an evolving AKI.

Our results indicate that urinary  $\pi$ -GST was expressed in all patients admitted to the ICU with a diagnosis of sepsis syndrome, whether or not they developed AKI as defined by AKIN. As all patients fulfilled the criteria for sepsis, the associated physiological derangements are known triggers for AKI. These results suggest that  $\pi$ -GST may identify patients with more subtle renal injury not identifiable with AKIN criteria. However, the ROC curves failed to demon-

strate that measurement of urinary levels of  $\pi$ -GST was specific or sensitive in predicting later development of AKI in this cohort. The study examined GST levels in the 48 hours after ICU admission, and was not designed to explore the value of GST as a marker later in the course of ICU stay.

We found that  $\alpha$ -GST levels in patients with AKI were similar to those in healthy control subjects. This raises concern about the value of  $\alpha$ -GST in diagnosing AKI caused by sepsis. Specific isoforms of GST are specific to the cytoplasm of cells of the renal tubules, with  $\alpha$ -GST and  $\pi$ -GST found in the proximal and distal tubules, respectively. Many studies have shown  $\alpha$ -GST to be a sensitive indicator of proximal tubular injury resulting from, for example, ischaemic and toxic insult.<sup>15</sup> However, there is no current histopathological evidence about the primary site of injury in sepsis-associated AKI,<sup>16</sup> and the increase we found in urinary  $\pi$ -GST levels without a change in  $\alpha$ -GST levels suggests that AKI in sepsis is a distal tubular injury. Further research is required to explore this possibility. In support of this, Ivanyi et al found that, in renal infections, bacteria tended initially to localise to the distal tubules.<sup>17</sup> Other recent research<sup>18</sup> showed a relationship between early  $\pi$ -GST elevation and Stage 3 AKI in a cohort of cardiac surgery patients who later required RRT, possibly reflecting a different pathophysiology within the cardiac surgery perioperative context. Interestingly, median  $\pi$ -GST elevations were lower for comparable stages of AKI.

A limitation of our study was that most of the AKI patients had Stage 1 AKI. We propose to undertake a prospective study of Stage 3 AKI by recruiting patients with sepsis syndrome who have established AKI at the time of ICU admission. An advantage of the current study protocol was the homogeneity of the patient population achieved by using a narrow admission diagnosis, resulting in fewer confounding factors. To our knowledge, no other study has examined urinary levels of  $\alpha$ -GST and  $\pi$ -GST in such a patient population.

The finding that urinary  $\pi$ -GST level was increased in all patients with a sepsis diagnosis, whether or not AKI criteria were subsequently met, may reflect subclinical kidney injury in patients with sepsis. Therapies such as large-volume resuscitation and inotropic and vasopressor agents targeting mean arterial pressure to maximise renal blood flow could be instituted earlier if AKI could be diagnosed at an earlier stage. In addition, a biomarker that could predict inevitable evolution to Stage 3 AKI might assist decision-making about earlier RRT and less aggressive volume and vasopressor therapies.

There is growing interest in the use of novel biomarkers to provide earlier diagnosis of AKI and monitor response to therapies, and the use of NGAL to diagnose AKI in patients with sepsis is showing particular promise. Zappitelli and

colleagues<sup>19</sup> examined urinary NGAL release in a heterogeneous population of critically ill children in a paediatric ICU, and found urinary NGAL to correlate with paediatric modified RIFLE criteria and to be a good early indicator of AKI. More specifically for patients with sepsis, Wheeler et al<sup>20</sup> recently demonstrated the usefulness of serum NGAL in the diagnosis of AKI. They compared NGAL release between children with systemic inflammatory response syndrome or sepsis, and a healthy control group. Although NGAL was a sensitive marker of AKI, it lacked specificity. Further study is required of these and other potential early markers of AKI.

In conclusion, urinary  $\pi$ -GST is an interesting new biomarker for AKI that may have practical therapeutic implications in the ICU and potential as a point-of-care test. Use of this urinary biomarker may help guide therapeutic interventions and indicate the success or failure of therapies in limiting further renal damage. If other studies confirm that more  $\pi$ -GST than  $\alpha$ -GST is released in AKI associated with sepsis, then the identification of the distal tubule as a significant site of injury could provide useful scientific insights.

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