Interobserver agreement for post mortem renal histopathology and diagnosis of acute tubular necrosis in critically ill patients

Neil J Glassford, Alison Skene, Maria B Guardiola, Matthew J Chan, Sean M Bagshaw, Rinaldo Bellomo and Kim Solez

ABSTRACT

Background: The renal histopathology of critically ill patients dying with acute kidney injury (AKI) in intensive care units of high income countries remains uncertain.

Methods: Retrospective observational assessment of interobserver agreement in the reporting of renal post mortem histopathology, and the ability of pathologists blinded to the clinical context to independently identify the presence of pre-mortem AKI from digital images of histological sections from 34 critically ill patients dying in teaching hospitals in Australia and Canada.

Results: We identified a heterogeneous cohort with a median age of 65 years (interquartile range [IQR], 56.5–77), APACHE II score of 27 (IQR, 19–33), and sepsis as the most common admission diagnosis (12/34; 35%). The most common proximate causes of death were cardiovascular (19/34; 56%) and respiratory (7/34; 21%) failure. AKI was common, with 23 patients (68%) developing RIFLE-F AKI, and 21 patients (62%) receiving renal replacement therapy. Structured reporting for tubular inflammation showed excellent agreement (kappa = 1), but no other subdomain demonstrated better than moderate agreement (kappa < 0.6). Only fair agreement (55.9% of cases; kappa = 0.23) was demonstrated on the diagnosis of moderate to severe acute tubular necrosis (ATN). Pathologist A predicted RIFLE-I or worse AKI with the diagnosis of ATN, with an overall accuracy of 61.8%; pathologist B predicted AKI with an accuracy of 35.3%.

Conclusions: Post mortem assessment of the renal histopathology in critically ill patients is neither robust nor reproducible; independent pathologists agree poorly on the diagnosis of ATN, and their structural assessment appears dissociated from ante-mortem renal function.

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Acute kidney injury (AKI) is common in critically ill patients, and is associated with increased mortality.[1,2] Despite clear information about the epidemiology of such loss of kidney function in critically ill patients,[1] information on the accompanying histopathological data are lacking, as renal biopsies are seldom performed in patients with AKI treated in the intensive care units (ICUs) of high income countries. The risk of such procedures in critically ill patients is high. Only rarely, and in specific situations, do the results lead to changes in management, with such biopsy findings reflecting selection bias in favour of a population with specific diagnostic issues.[3] Moreover, the limited sampling associated with biopsies may induce speculation, and the reporting of an overall diagnostic impression rather than detailed findings. Our current understanding of structural changes during AKI in critically ill patients in high income countries is therefore based on post mortem studies.[4-6] However, it is unknown whether such post mortem assessment of renal histopathology in ICU patients is robust, reproducible and correlates with functional changes.

Acute tubular necrosis (ATN) — often used interchangeably with acute tubular injury — is used in pathology to describe the constellation of morphological features caused by destruction or injury of the renal tubular epithelium due to ischaemia or less common toxins.[7,8] Features include necrosis of epithelial cells as well as a range of less severe degenerative and regenerative changes. The assumption that ATN is the morphological correlate of AKI in sepsis has been challenged, but human studies have been limited by a small sample size and methodological differences.[9]

Accordingly, we conducted a retrospective observational study of the clinical and histopathological records of patients dying in two university-affiliated ICUs in two different countries who underwent subsequent autopsy. We aimed to assess the agreement between independent pathologists scoring post mortem changes in renal histopathology using a semi-quantitative scoring system. We also aimed to determine if the histological diagnosis of ATN, made by pathologists blinded to both clinical information and to each other's assessment, was able to reliably identify patients who had significant AKI at the time of death.

Methods

Ethical approval

Local research ethics committees at the Austin Hospital, Melbourne, Australia, and the University of Alberta Hospital, Edmonton, Canada, approved this study before commencement and waived the need for informed consent.

Study design

We conducted a dual-centre retrospective analysis of critically ill patients dying in tertiary referral university-affiliated ICUs who underwent subsequent autopsy.

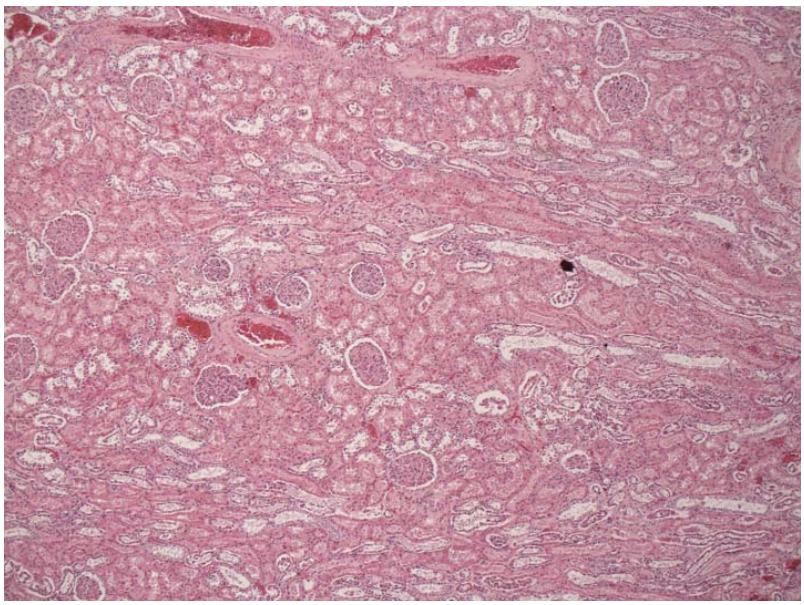
Inclusion and exclusion criteria

Renal histological material and images were obtained from patients who had died in the ICU at either site and had undergone post mortem examination. Patients were eligible for inclusion in the study if the pathologists agreed that the tissue preparation was of sufficient quality for an assessment to be made. Material from children under the age of 18 years and patients requiring chronic haemodialysis were excluded.

Data sources and definitions

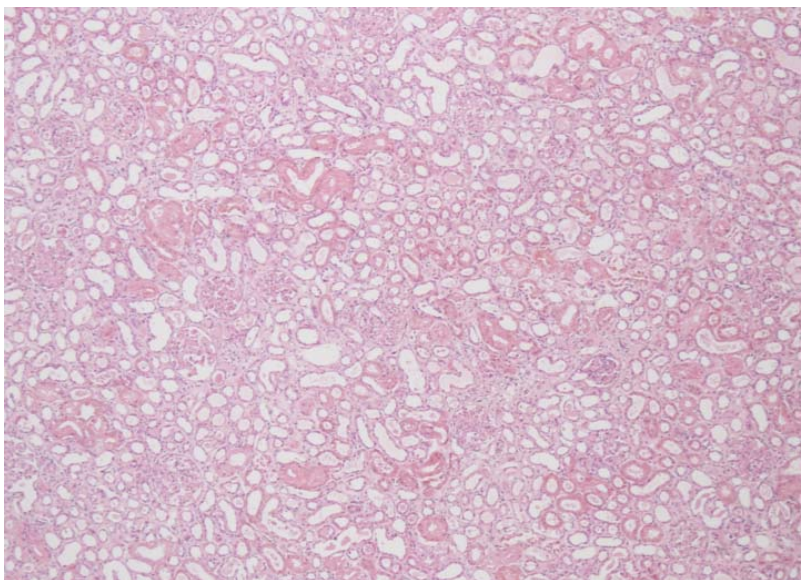
Data on demographics, therapeutic interventions, nephrotoxin and vasoactive exposure, and pre-existing medical conditions were obtained using local intensive care reporting databases and hospital records. Laboratory databases provided biochemical and haematological indices. Death certification and record review provided the proximate, contributory and underlying causes of death. Extended definitions are available in the online supplementary Appendix (supplementary Appendix, Additional methods; online at cicm.org.au/Resources/Publications/Journal).

Figure 1. Images of renal necroscopic tissue as used for histopathological assessment (x 40 magnification)*



* Both pathologists agreed that there was no evidence of acute kidney injury.

Figure 2. Images of renal necroscopic tissue as used for histopathological assessment (x 40 magnification)*



* Classical changes of acute tubular necrosis, as agreed by both pathologists, in a patient who developed RIFLE-F (risk of renal dysfunction, injury to the kidney, failure of kidney function, loss of kidney function, and end-stage kidney disease) AKI (acute kidney injury) requiring continuous renal replacement therapy on a background of hepatorenal failure, chronic liver disease and chronic cardiovascular disease.

We determined the presence and severity of AKI using both the creatinine and urinary definitions of the RIFLE criteria (risk of renal dysfunction, injury to the kidney, failure of kidney function, loss of kidney function, and end-stage kidney disease) (supplementary Appendix, Table E1).[10] We took the lowest creatinine obtainable within 12 months preceding hospital admission as the baseline creatinine, if available; we otherwise used creatinine on admission to hospital. We took the highest creatinine during the hospital admission to represent the peak serum creatinine value. Significant AKI was defined as RIFLE-I or -F AKI, or receipt of renal replacement therapy.

Renal histopathological material

Paraffin sections of renal material were taken as standard. Selected high-powered and low-powered digital images of the sections were taken, which were considered by each pathologist to be representative of the tissue being examined (Figure 1 and Figure 2).

Semi-quantitative histological scoring

Two pathologists from tertiary referral institutions, who remained blinded to peri-mortem clinical information, assessed the images of renal samples. The pathologists were asked to assess the degree of autolysis, the features of which overlap with milder forms of acute tubular injury. After such initial assessment, four additional histological domains likely to be indicative of underlying AKI, as well as measures of chronic damage, were assessed and scored using a semi-quantitative approach. Each domain was subdivided into, and scored on, relevant subdomains, with 0 = no, 1 = mild, 2 = moderate, 3 = severe abnormalities (Table 1). Renal samples were histologically categorised as "no or mild ATN" or "moderate to severe ATN" based on the constellation of above features. Each pathologist at the home site provided a full report and scoring of renal post mortem tissue from each patient. In addition, each pathologist obtained several digital images of key areas of the kidney for each patient. Such multiple images were then sent via an independent clinical investigator blinded to the report (RB and SMB) to the other pathologist for assessment. Each pathologist was blinded to any interpretation or reporting by the other, and to any clinical information regarding the hospital or intensive care admission.

Data analysis

Statistical analysis was performed using STATA version 13 (StataCorp, College Station, Texas, United States). Descriptive statistics were generated and compared as appropriate. The ability of the histological identification of moderate to severe ATN to diagnose significant AKI was summarised using standard summary statistics. Interobserver agreement was assessed using the kappa statistic. Interobserver agreement was assessed on each subdomain using weighted kappa. Kappa or weighted kappa values = 0.81–1 were taken to indicate excellent agreement; kappa = 0.61–0.8, substantial agreement; kappa = 0.41–0.6, moderate agreement; kappa = 0.21–0.4, fair agreement; and kappa = 0–0.2, only slight agreement.

Table 1. Semi-quantitative histopathological assessment tool

Domain	Subdomain	Scoring
Tubules	Dilatation	0 = no change
	Thinning	1 = mild abnormalities
	Cell sloughing	2 = moderate abnormalities
	Presence of casts	3 = severe abnormalities
	Vacuolation	
	Apoptosis	
	Regeneration	
	Tubular inflammation	
Interstitium	Inflammation	
	Oedema	
	Fibrosis/atrophy	
Vascular	Vasa recta	
	Arteries	
	Arterioles	
Glomeruli	Parietal epithelial changes	
	Sclerosis	
Autolysis		

Results

Patients

We analysed the clinical features and tissue samples of 34 patients, 20 (59%) male, with a median age of 65 years (interquartile range [IQR], 56.5–77), and a median APACHE (Acute Physiology and Chronic Health Evaluation) II score of 27 (IQR, 19–33) (Table 2). Sepsis was the most common admitting diagnosis (12/34; 35%), and hypertension (18/34; 53%) and cardiovascular disease (18/34; 53%) the most common comorbidities. The most common proximate causes of death were cardiovascular (19/34; 56%) and respiratory (7/34; 21%) failure. Within 24 hours of death, the majority of patients were ventilated (27/34; 79%) and being treated with vasoactive medications (24/34; 71%). Detailed information on all patients is provided in the online Appendix (online Appendix, Table E2).

Renal function and acute kidney injury

The median pre-morbid serum creatinine in the cohort was 69 µmol/L (IQR, 83–107), with a median creatinine of 85 µmol/L (IQR, 135–223) on ICU admission. Overall, 31 patients (91%) were exposed to one or more nephrotoxins during their hospital admission. AKI was common, with 26 patients (77%) developing at least RIFLE-I AKI, and 23 patients (68%) developing RIFLE-F AKI. Finally, 21 patients (62%) received renal replacement therapy (Table 3).

Histopathological assessment and semi-quantitative scoring

The median time from patient death to autopsy and paraffin fixation of the renal tissue was 22.5 hours (IQR, 15–34).

Scores for tubular inflammation showed excellent agreement (kappa = 1). No other subdomain, however, demonstrated better than moderate agreement between pathologists (kappa < 0.6). Moreover, only slight agreement (kappa < 0.2) was demonstrated in the scoring of tubular sloughing, apoptosis or regeneration, interstitial oedema, or glomerular sclerosis.

Table 2. Demographic characteristics of the patient cohort

Characteristics	Value*
Age (years)	65 (56.5–77)
Sex (male)	20 (59%)
Height (*n* = 24/34) (cm)	169 (160–173)
Weight (*n* = 29/34) (kg)	77 (65–87)
Admitting diagnosis	
Sepsis, any source	12 (35%)
Cardiovascular surgery, failure or arrest	7 (21%)
Liver/biliary disease	6 (18%)
Surgical	9 (27%)
Comorbidities	
Cardiovascular disease	18 (53%)
Hypertension	18 (53%)
Respiratory disease	9 (27%)
Liver disease	9 (27%)
Diabetes mellitus	8 (24%)
Malignancy	13 (38%)
Immunocompromised	5 (15%)
Illness severity	
APACHE II score	27 (19–33)
SAPS 2 score	41 (27–60)
At death	
Ventilated	27 (79%)
Vasoactive medication	24 (71%)
Multiple organ failure	23 (68%)
Withdrawal of care	16 (47%)
Proximate cause of death	
Cardiovascular	**19 (56%)**
Respiratory	7 (21%)
Neurological	4 (12%)
Metabolic	3 (9%)
Malignancy	1 (3%)
Contributory causes of death	
Number	2 (1–3)
Cardiovascular	14 (41%)
Respiratory	8 (24%)
Neurological	3 (9%)
Gastrointestinal	18 (53%)
Malignancy	11 (32%)
Renal	4 (12%)
Metabolic	5 (15%)

APACHE = Acute Physiology and Chronic Health Evaluation. SAPS = Simplified Acute Physiology Score. * Values are median (interquartile range) or *n* (%).

Table 3. Creatinine, acute kidney injury (AKI) and nephrotoxin exposure

Renal characteristics	Value*
Serum creatinine values	
Pre-morbid (*n* = 31/34), µmol/L	69 (83–107)
On hospital admission, µmol/L	89 (113–188)
On ICU admission, µmol/L	85 (135–223)
Within 24 hours of death (*n* = 33/34), µmol/L	149 (99–224)
AKI	
RIFLE-I	26 (77%)
RIFLE-I creatinine criteria	19 (56%)
RIFLE-I urinary criteria	19 (56%)
RIFLE-F	23 (68%)
RIFLE-I creatinine criteria	11 (32%)
RIFLE-I urinary criteria	18 (53%)
Patients presenting to hospital with peak AKI	8 (31%)
Time from hospital admission to peak AKI (days)	1.7 (0–7.4)
RRT	21 (62%)
Nephrotoxin exposure	
Exposure to one or more nephrotoxins	31 (91%)
Intravenous contrast	15 (44%)
Aminoglycosides	6 (18%)
Amphotericin	2 (6%)
Myoglobin/rhabdomyolysis	6 (18%)
Calcineurin inhibitors	1 (3%)
Diuretics	22 (65%)
Starches	1 (3%)
Others	7 (21%)

ICU = intensive care unit. RIFLE = risk of renal dysfunction, injury to the kidney, failure of kidney function, loss of kidney function, and end-stage kidney disease. RRT = renal replacement therapy. * All values median (interquartile range) or *n* (%) unless otherwise noted.

Identifying acute kidney injury from a diagnosis of acute tubular necrosis

Pathologist A made 21 diagnoses of moderate to severe ATN, while Pathologist B made only six. Pathologists A and B agreed on the diagnosis of moderate to severe ATN in 55.9% of cases, with a kappa = 0.23 indicating only fair agreement.

The diagnosis of moderate to severe ATN made by pathologist A predicted RIFLE-I or worse AKI with a sensitivity of 65.4% (95% confidence interval [CI], 44.4–82.8%), a specificity of 50% (95% CI, 15.7–84.3%), with a positive predictive value (PPV) of 81% (95% CI, 58.1–94.6%) and a negative predictive value (NPV) of 30.8% (95% CI, 9.1–61.5%). Overall, accuracy was 61.8%.

The diagnosis of moderate to severe ATN made by pathologist B predicted RIFLE-I or worse AKI with a sensitivity of 19.3% (95% CI, 6.6–39.4%), a specificity of 87.5% (95% CI, 47.4–99.7%), with a PPV of 83.4% (95% CI, 35.9–99.6%) and a NPV of 25% (95% CI, 10.7–44.9%). Overall accuracy was 35.3%.

Discussion

Key findings

We assessed the interobserver agreement between two experienced renal pathologists, blinded to each other's interpretation of the histopathological findings and to any clinical information, and the accuracy with which their diagnosis of moderate to severe ATN could predict the presence of moderate to severe AKI in a cohort of critically ill patients, from two teaching hospitals in Canada and Australia, who had died in ICU and who had suitable post mortem tissue. We found variable, but generally low agreement between pathologists across almost all histological domains. Moreover, we found that agreement on the diagnosis of moderate to severe ATN was fair at best. Finally, we found great variation in both specificity and sensitivity for the prediction of the presence of moderate to severe clinical AKI by diagnosis of moderate to severe ATN, with an overall accuracy level of between 35.3% and 61.8%.

Relationship to previous studies

There is little information available on the histopathological features of post mortem kidneys in critically ill patients from high income countries treated in a contemporary environment. In a unique population of 19 patients with a greater than 30% incidence of disseminated intravascular coagulation, from a single French ICU, receiving immediate post mortem assessment in the setting of septic shock and anuria, acute tubular injury, inflammatory infiltrate, and apoptosis were prominent findings.[5] However, this highly selected population was not reported by pathologists blinded to clinical data, did not require a detailed specific and semi-quantitative assessment of all aspects of histological findings, did not allow for assessment of interobserver agreement, and did not assess the pathologist's ability to identify the degree of functional loss on the basis of structural changes. Moreover, the findings reported in this highly selected cohort are at odds with recent data describing the renal histopathology of experimental septic shock.[11] They are also divorced from extensive animal data from about 1000 animals and 100 experimental studies.[12] This level of disagreement highlights the potentially subjective nature of histological diagnosis and the uncertainty about the underlying histopathology in critically ill patients with severe AKI in such contexts.

In addition, the seeming lack of correlation between histological significant ATN and significant AKI on post mortem histopathological assessment, the limited ability to diagnose ATN in a reproducible manner, and the failure to predict renal function from histological features are all in keeping with the view that, in many ICU patients, AKI may represent a functional phenomenon.[11]

Our findings are consistent with the wider literature suggesting that poor interobserver agreement may be a pervasive phenomenon in histopathological diagnosis. For example, histopathological findings remain relatively subjective, with wider than expected interobserver variability, even when using well validated scoring systems, such as the Banff working classification of kidney transplant pathology.[13,14] In a single-centre study involving five histopathologists, overall agreement varied from 0.48 to 0.7 depending on the domain examined.[15] In a study of 22 major transplant centre laboratories, reproducibility was shown to be relatively poor, varying from a kappa = 0.05 for assessment of neutrophil presence, to kappa = 0.53 for the number of glomeruli present, consistent with our findings.[14] In a recent American study of almost 7000 case diagnoses of breast pathology by 115 histopathologists, there was only an overall 75.3% concordance rate, though this varied from 48% for cases of atypia to 96% for invasive carcinoma. Pathologists with lower weekly case volumes, or those from small or non-academic settings were more likely to disagree with reference diagnoses.[16]

Diagnostic confidence and interobserver agreement increase significantly with the amount of clinical information provided to pathologists diagnosing skin malignancies, though, in an international cohort, overall agreement only increased from moderate (kappa = 0.57) to substantial (kappa = 0.67).[17]

Finally, it remains uncertain, whether post mortem findings reflect the true histopathology immediately ante-mortem. Logically, the hours preceding death are associated with a period of renal hypoperfusion and tissue hypoxia; this would likely lead to additional renal histological injury above and beyond any pre-existing injury. Irrespective of such considerations, however, it is logically impossible that the histological findings present in the pre-mortem hours would ever be more severe than those seen post mortem.

Study implications

Our study implies that usual practice post mortem assessment of the renal histopathology in critically ill patients dying in the ICU of a teaching hospital in high income countries is neither robust nor reproducible, nor is our ability to make the diagnosis of ATN at post mortem. This suggests that the use of ATN should be restricted to histopathological, not

clinical discussion and diagnostics. Finally, as pathologists are unable to derive any sense of likely loss of function from assessing tissue structure, our study implies that renal function and structure may be dissociated in at least some mortally ill ICU patients.

Strengths and limitations

To our knowledge, our study provides novel information on the agreement between pathologists blinded to the clinical context and to each other's report in describing the post mortem histopathological changes associated with AKI in the critically ill. We included patients from two different centres on different continents. We adopted a detailed and structured assessment of all elements of renal tissue visible to the pathologist, yet preserved the ability to provide an overall impression on the presence of the diagnosis most commonly believed to represent the histopathological substrate of severe AKI. For the first time, we also assessed the ability of pathologists to predict the presence or absence of major loss of function by their assessment of the renal histopathology. The demonstration that such prediction is of limited accuracy provides more indirect evidence that a significant component of loss of glomerular filtration in critically ill patients may be functional.

Our study, however, has several limitations. First, it only involved two centres, limiting external validity. However, all previous studies have been single centre in nature, and our two centres were from two different countries. Second, the study population was small. However, to our knowledge, this cohort of patients was close to twice the size of two previous post mortem studies in critically ill patients,[4,5] and similar in size to the largest post mortem study of renal histopathology so far.[6] Of note, however, all such studies have been confined to patients with sepsis or septic shock, thus our study is, to our knowledge, also the first in a heterogeneous population of critically ill patients. Third, post mortem delays were responsible for autolysis, which produces changes difficult to distinguish from pre-mortem changes.[18] Features that have been suggested as more specific to pre-mortem ATN — including apoptosis, regenerative epithelial changes, mitoses and inflammation[19] — were rarely seen in our study, most likely due to the post mortem delays (mean, 22.5 hours). While the most severely autolysed cases were excluded from the study, the pathologists differed in their willingness to diagnose ATN in the presence of autolytic changes, which is reflected in differences in number of diagnoses and specificity. We only assessed for the presence of apoptosis using histopathological examination. However, this is the typical assessment applied under routine non-research settings. Other studies have assessed for apoptosis in a more structured way using specific and more sensitive techniques, but have reported contradictory findings.[4-6] Fourth, we assessed agreement between two specific experienced academic renal pathologists in teaching hospitals. One pathologist (KS) is an international expert in the field of AKI and ATN histopathology; the other (AS) spent her sabbatical in the institution where KS works. Both have similar educational backgrounds. Although we think it unlikely, other pathologists might have achieved a greater level of agreement, though interobserver reproducibility of renal biopsy findings has been shown to be poor in other settings,[14] and pathologists who have worked together are more likely to demonstrate a greater degree of reproducibility than those working in separate institutions.[13]An additional limitation was our use of high fidelity digital images instead of paraffin sections. This approach facilitated blinding, and by each pathologist selecting the most representative fields, actually may have increased the likelihood of agreement through selection bias. Finally, the inability of pathologists to guess renal functional status is biologically plausible and likely to reflect mounting experimental evidence that in septic AKI, histology may be dissociated from function.[11]

Conclusions

In summary, post mortem assessment of the renal histopathology of critically ill patients dying in the ICU of teaching hospitals in high income countries is neither robust nor reproducible. Moreover, as independent pathologists show low levels of agreement on the post mortem histopathological diagnosis of ATN, because of the confounding effects of post mortem autolysis, such diagnosis is also neither robust not reproducible. Finally, as pathologists are unable to estimate likely pre-mortem loss of function from their histopathological assessment, renal function and structure appear dissociated in critically ill patients.

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Study concept and design: Glassford, Bellomo, Skene, Bagshaw and Solez.

Acquisition of data: Glassford, Chan, Guardiola, Bellomo, Skene, Bagshaw and Solez.

Analysis and interpretation of data: Glassford, Bellomo, Skene, Bagshaw and Solez.

Drafting of the manuscript: Glassford, Bellomo and Solez.

Critical revision of the manuscript for important intellectual content: Glassford, Chan, Guardiola, Bellomo, Skene, Bagshaw and Solez.

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Competing interests

None declared.

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Appendix

This appendix was part of the submitted manuscript and has been peer reviewed. It is posted as supplied by the authors

Additional methods

Defining comorbidities

Pre-existing cardiovascular disease was defined by the presence of any of the following conditions on chart review: stroke, transient, angina, claudication, PCI, coronary bypass and recognized, silent myocardial infarction, heart failure (≥NYHA Class III Symptoms), or peripheral vascular disease and/or bypass.

Hypertension was defined by the use of anti-hypertensive medication on chart review.

Respiratory disease was defined by presence of chronic restrictive, obstructive or vascular respiratory disease causing severe exercise limitations or documented chronic hypoxemia, hypercapnia, secondary polycythemia or pulmonary hypertension on chart review.

Liver disease was defined by presence of biopsy proven cirrhosis or documented evidence of portal hypertension (i.e. variceal bleeding, ascites, encephalopathy) on chart review.

Diabetes mellitus was defined by use of insulin or oral hypoglycemic agents on chart review.

Solid organ/haematologic malignancy was defined by the presence of any documented metastatic solid organ tumour or presence of any form of hematologic malignancy on chart review.

Immunocompromise was defined by the presence of advanced disease sufficient to suppress resistance to infection (i.e. malignancy, AIDS) or therapy that suppresses resistance to infection (i.e. chemotherapy, steroids) on chart review.

Defining causes of death

Proximate causes of death were defined as

Neurological proximate causes of death included brain death; hypoxic encephalopathy; intracranial haemorrhage; ischaemic stroke; and other.
Cardiovascular proximate causes of death included primary arrhythmia; refractory cardiogenic shock (including pulmonary oedema); cardiac tamponade; hypovolaemia (including uncontrollable bleeding); septic shock; massive pulmonary embolism; anaphylaxis; and other.
Respiratory proximate causes of death included refractory hypoxia due to adult respiratory distress syndrome (ARDS); chronic obstructive pulmonary disease (COPD); asthma; pulmonary hemorrhage; pneumothorax; and other.
Metabolic proximate causes of death included hypoglycemia; hyperkalemia; hypothermia; liver failure; and other.
Patients dying with multiple organ failure or following withdrawal of therapy were also identified.

Underlying causes of death were defined as

Neurological underlying causes of death included cerebrovascular accident; dementia; subarachnoid hemorrhage; neurological infection; spinal cord injury; seizures.

Cardiovascular underlying causes of death included ischemic heart disease; hypertension; abdominal or thoracic aortic aneurysm; peripheral vascular disease; coronary artery disease; congestive cardiac failure.

Respiratory underlying causes of death included COPD; asthma; pulmonary fibrosis; active tuberculosis infection; pneumonia; pulmonary embolism.

Gastrointestinal underlying causes of death included gastrointestinal tract cancer; hepatic failure; cirrhosis; gastrointestinal bleeding; gastrointestinal obstruction; pancreatitis; inflammatory bowel disease; anorexia; obesity.

Metabolic underlying causes of death included diabetes mellitus; hypothyroidism; adrenal insufficiency.

Renal underlying causes of death included chronic kidney disease (CKD); pyelonephritis; renal artery stenosis; renal cell carcinoma.

Hematological underlying causes of death included coagulopathy.

Other noted underlying causes of death included pregnancy and disseminated malignancy.

Table E1. RIFLE classification of acute kidney injury

Grade	Serum Creatinine criteria (µmol/l)	Urinary Output Criteria
Risk	↑ serum creatinine x 1.5	UO < 5ml/kg/h for ≥ 6h
Injury	↑ serum creatinine x 2	UO < 5ml/kg/h for ≥ 12h
Failure	↑ serum creatinine x 3 or serum creatinine ≥ 350 with rise of ≥ 44	UO < 3ml/kg/h for ≥ 24h or Anuria for ≥ 12h
Loss	Persistent need for RRT for more than 4 weeks	
ESKD	End Stage Kidney Disease at more than 3 months	

UO: urine output; RRT: renal replacement therapy

Table E2. Individual patient demographics, renal status and nephrotoxin exposure

Patient	Age	Sex	Wt kg	Ht cm	ICU Diagnosis	APACHE II	SAPS 2	Baseline Cr	Baseline Urea	Peri-mortem Cr	Peri-mortem Urea	Peak AKI	Time to Peak AKI	CRRT	CRRT at death	Nephrotoxin Expisure
ATN01	78	M	68	165	Cardiac surgery	21	36	136	13.3	221	6.8	RIFLE-F	11.17	Yes	Yes	Contrast, Rhabdomyolysis, Diuretics, Other
ATN02	86	F	54	145	Septic Shock	21	51	56	3.5	175	21.7	RIFLE-F	1.96	No	No	Diuretics, Other
ATN03	51	F	50	154	Decompensated CLD	33	76	68	2.6	101	7.1	RIFLE-F	8.35	Yes	Yes	Diuretics
ATN04	49	M	46	175	Pneumonia	19	48	83	4.5	93	9.3	RIFLE-I	29.41	No	No	Contrast, Diuretics
ATN05	56	F	90	162	Hepatorenal Failure	15	54	94	5.9	262	19.2	RIFLE-F	0.00	Yes	Yes	None
ATN06	78	M	78	164	Aortic Surgery with CPB	17	41	114	10.8	156	5.4	RIFLE-F	0.75	Yes	Yes	Diuretics
ATN07	79	M	79	171	Ischaemic Stroke	30	67	132	13.4	144	16.1	None		No	No	Contrast, Rhabdomyolysis, Diuretics
ATN08	55	M	98	177	Pneumonia	27	36	106	3	107	12.5	RIFLE-F	2.45	Yes	Yes	Contrast, Aminoglycosides, Diuretics, Other
ATN09	70	M	67	173	post-VATS	28	66	90	7.3	61	12.5	None		No	No	Contrast, Diuretics
ATN10	69	M	87	179	Post-operative	16	26	107	5	131	3.9	RIFLE-F	3.42	Yes	Yes	Aminoglycosides, Rhabdomyolysis
ATN11	81	M	78	170	Cardiac surgery	24	47	92	6.6	212	6.8	RIFLE-I	5.08	No	No	Contrast, Aminoglycoside, Rhabdomyolysis, Diuretics, Other
ATN12	56	M			Biliary Sepsis	24	38	64	2.6			None		No	No	Contrast
ATN13	22	F	50	156	Staphylococcal Septicaemia	19	33	136	6.6	153	7.4	RIFLE-F	2.41	Yes	Yes	Contrast, Aminoglycosides, Diuretics
ATN14	65	M	50	173	Post-laparotomy	14	23	65	3.4	66	3.3	RIFLE-I	0.00	No	No	Contrast, Aminoglycosides, Diuretics
ATN15	53	M	83	180	Septic Shock	15	43	93	8	412	28.4	RIFLE-F	0.00	Yes	Yes	Calcineurin inhibitors, Diuretics
ATN16	67	F			Septic Shock	41	95	71	1.7	153	5.5	RIFLE-F	0.30	Yes	Yes	Rhabdomyolysis
ATN17	79	M	80	172	Hyperkalaemia	27	35	79	13.2	239	11.1	RIFLE-F	0.00	Yes	No	Other
ATN18	73	M			Cardiac Arrest	38	63	73	4.7	79	4.3	None		No	No	None
ATN19	77	M	83	173	Aspiration Pneumonia	21	40	77	4.4			RIFLE-F	0.00	No	No	Diuretics, Other
ATN20	82	F	87		Aneurysme repaired	38	83	140	12.2	260	16.3	RIFLE-F	21.51	Yes	No	Contrast, Diuretics
ATN21	64	M	70	172	Pneumonia	13	38	55	6.4	104	17.4	None		No	No	Diuretics
ATN22	76	M	65	168	Tracheoesophageal fistula repareid	19	27	50	3	117	26.5	RIFLE-F	38.26	Yes	No	Aminoglycosides, Diuretics
ATN23	65	F	69	160	Retroperitoneal haematoma	31	59	128	7.8	139	3.6	RIFLE-F	0.00	Yes	Yes	Contrast
ATN24	58	F			Fulminant liver failure	21	52	70	0.9	233	11.5	RIFLE-F	1.33	Yes	Yes	Amphotericin, Diuretics
ATN25	53	M	70	169	Fulminant liver failure	50	84	94	2.6	86	4.9	RIFLE-F	0.14	Yes	Yes	Amphotericin
ATN26	72	M	90		Thrombosed bypas axillo-bifemoral	30	60	150	9.9	310	12.9	RIFLE-F	0.96	Yes	Yes	Contrast, Rhabdomyolysis, Diuretics
ATN27	59	F	75		Hepatic encephalopathy	47	99	84	13.2	159	18.9	RIFLE-F	8.21	Yes	Yes	Diuretics
ATN28	60	F	100	142	Hypoxemic Respiratory Failure	23	8	67		94	8.2	None		No	No	Contrast
ATN29	84	M	77		Cardiac surgery	38	20	71	7.8	235	39.5	RIFLE-F	39.00	Yes	Yes	Diuretics
ATN30	77	0	58	169	Cardiogenic Shock	32				369	26.3	RIFLE-F	0.00	Yes	Yes	Diuretics
ATN31	59	1			Pneumonia		16	83	6.2	154	15.1	RIFLE-F	3.00	Yes	Yes	Contrast

ATN32	48	0	110	160	Cardiogenic Shock	41	21			114	11.3	None		No	No	Diuretics, Starch
ATN33	58	0	93.4		Hemorrhagic Shock	28	7	66	5.6	79	12.4	None		No	No	None
ATN34	59	0	49	159	Septic Shock	37	22			90	4.3	RIFLE-F	0.00	Yes	Yes	Contrast

APACHE II=acute physiology and chronic health evaluation score version II; SAPS 2=simplified acute physiology score version 2; AKI = acute kidney injury
CRRT=continuous renal replacement therapy

Table E3. Individual patient peri-mortem characteristics and causes of death

Patient	Peri-mortem characteristics										Cause of Death			
	IPPV	Vasoactives	HCT	WCC	Bili	FiO2	PaO2	CK	Lactate	24h FB	Proximate Cause of Death	Underlying Causes of Death	MOF	Withdrawal
ATN01	Yes	Yes	0.22	20.6	65	0.7	77	2,221	9.96	1041	Refractory Shock	IHD, HT, PVD, COPD, DM	Yes	No
ATN02	Yes	Yes	0.33	18.9	13	0.5	77	20	4.5	1400	Septic Shock	IHD, CHF	Yes	No
ATN03	Yes	Yes	0.24	41.8	472	0.4	83		6.57	21	Pneumonia	PHT, CLD, Coagulopathy	Yes	Ceiling of Rx
ATN04	No	No				1				-2200	Refractory hypoxia	Metastatic RCC	Yes	Yes
ATN05	No	Yes	0.29	35.7	226	1	78	71	11		Refractory Shock	CHF, ALF	Yes	No
ATN06	Yes	Yes	0.23	6.4	36	1	80		3.48	2800	Bleeding	GIB	Yes	No
ATN07	Yes	Yes	0.3	24.5	14	0.6	62	2,332	2.75	312	Ischaemic Stroke	CVA, IHD	No	Yes
ATN08	Yes	Yes	0.23	3.4	530	0.5	85	37	10.6	-1500	Intracranial Haemorrhage	CVA, HL, ALF	Yes	No
ATN09	Yes	No	0.27	12.1	13	0.8	64	88	8.8	1800	Pneumonia	Pneumonia, NHL	No	Yes
ATN10	Yes	Yes	0.2	12	66	1	79	135,736	24.7	4300	Refractory Shock	CES, ALF, Renal infarct	Yes	No
ATN11	Yes	Yes	0.28	5.2	5	1	122	1,891	13	6500	Refractory Shock	IHD, GIB	Yes	No
ATN12	No	No									Metastatic CRC	Cancer, SBO	Yes	No
ATN13	Yes	Yes	0.29	36.8	386	0.7	71		22.8	1690	Refractory Shock	Sepsis, CLD, Pericarditis	Yes	Ceiling of Rx
ATN14	No	No									Refractory Shock	Malignancy, GIB, Pneumonia	No	No
ATN15	Yes	Yes	0.36	19.6	271	1	66	623	22.9	2500	Septic Shock	ALF, cholangitis	Yes	Yes
ATN16	Yes	Yes	0.2	4.9	68	0.9	88	13,910	14.3	6000	Septic Shock	ALF	Yes	Yes
ATN17	No	No	0.3	37.1	11						Other	Metastatic RCC	No	No
ATN18	Yes	Yes	0.14	4.9	18	0.7	92	330	5.4	1100	Primary arrhythmia	Cancer	No	Yes
ATN19	No	No	0.34	12.1							Aspiration Pneumonia	HL	No	No
ATN20	Yes	Yes	0.23	17.6	81	0.7	62	604	2		Septic shock	IHD, DM	No	No
ATN21	Yes	No	0.29	16.6	11	1	57		5	1285	Refractory hypoxia	Cancer, COPD	No	Yes
ATN22	No	No	0.28	11.9	15	0.5	72		0.93	316	Other	Cancer	Yes	Yes
ATN23	Yes	Yes	0.25	3.2	34	0.5	95	144,515	10.6	5960	Refractory shock	IHD, ALF	Yes	No
ATN24	Yes	Yes	0.23	14.3	391	0.6	69		3.8	1854	Septic shock	ALF, CLD, Pneumonia	Yes	No
ATN25	Yes	Yes	0.19	22	263	0.4	173		13.3	-2200	Liver Failure	ALF, CLD, GIB	Yes	Yes

ATN26	Yes	Yes	0.22	10.7	34	1	92	83848	11.31	841	Septic shock	PVD	Yes	No
ATN27	Yes	Yes	0.29	12.3	336	0.6	62	82	4.58	2469	Hypoxic encephalopathy	ALF, IHD, CLD	Yes	Yes
ATN28	Yes	No	0.27	23.2	16	1	56	95	2.6		Refractory hypoxia	COPD, Malignancy	No	Yes
ATN29	Yes	Yes	0.24	12	50	0.7	80		11.7	1883	Refractory shock	IHD, CHF	Yes	Yes
ATN30	No	No	0.21	26.4		1	51	90			Other-Pneumonia	Pneumonia, CKD, HT	Yes	Yes
ATN31	Yes	Yes	0.24	3.9	26	1	76	21	0.9	-1056	Ischemic stroke	Pneumonia, Malignancy	Yes	Yes
ATN32	Yes	Yes	0.2	20.8	39	1	57		23.2		Refractory shock	IHD, CHF	No	Yes
ATN33	Yes	Yes	0.25	8.3		1	547		14.4		Hypovolemia	IHD, GIB	No	Yes
ATN34	Yes	Yes	0.19	3.1	75	1	63	708	22		Septic shock	Malignancy	Yes	No

IHD=ischemic heart disease, HT=hypertension, PVD=peripheral vascular disease, COPD=chronic obstructive pulmonary disease, DM=diabetes mellitus, CHF=congestive heart failure, PHT=pulmonary hypertension, CLD=chronic liver disease, RCC=renal cell carcinoma, ALF=acute liver failure; GIB = gastrointestinal bleeding, CVA=cerebrovascular accident, HL= Hodgkin's lymphoma, NHL=non-Hodgkin's lymphoma; CES=cholesterol emboli syndrome, SBO=small bowel obstruction.

Table E4. Individual patient semi-quantitative scores and diagnostic agreement between pathologists

Patient	Peak AKI	CRRT at death	Nephrotoxin Exposure	Post-mortem delay	Pathologist A			Pathologist B			Agreement
				(hrs)	Autolysis score	ATN SQS	ATN?	Autolysis score	ATN SQS	ATN?	
ATN01	RIFLE-F	Yes	Contrast, Rhabdomyolysis, Diuretics, Other	22	3	16	Yes	2	11	No	No
ATN02	RIFLE-F	No	Diuretics, Other	16	1	17	Yes	2	15	Yes	Yes
ATN03	RIFLE-F	Yes	Diuretics	18	2	7	Yes	1	1	No	No
ATN04	RIFLE-I	No	Contrast, Diuretics	28	1	11	Yes	1	6	Yes	Yes
ATN05	RIFLE-F	Yes	None	4	0	12	Yes	0	16	Yes	Yes
ATN06	RIFLE-F	Yes	Diuretics	20	1	13	yes	2	11	No	No
ATN07	None	No	Contrast, Rhabdomyolysis, Diuretics	28	3	8	yes	3	12	No	No
ATN08	RIFLE-F	Yes	Contrast, Aminoglycosides, Diuretics, Other	7	2	11	yes	1	10	No	No
ATN09	None	No	Contrast, Diuretics	19	2	8	no	1	5	No	Yes
ATN10	RIFLE-F	Yes	Aminoglycosides, Rhabdomyolysis	15	3	10	yes	3	9	No	No
ATN11	RIFLE-I	No	Contrast, Aminoglycoside, Rhabdomyolysis, Diuretics, Other	11	1	12	no	2	8	No	Yes
ATN12	None	No	Contrast	34	3	7	no	3	5	No	Yes
ATN13	RIFLE-F	Yes	Contrast, Aminoglycosides, Diuretics	7	2	14	yes	1	17	Yes	Yes
ATN14	RIFLE-I	No	Contrast, Aminoglycosides, Diuretics	37	3	3	no	1	10	No	Yes
ATN15	RIFLE-F	Yes	Calcineurin inhibitors, Diuretics	24	2	8	yes	2	8	No	No
ATN16	RIFLE-F	Yes	Rhabdomyolysis	25	3	2	no	3	5	No	Yes
ATN17	RIFLE-F	No	Other	27	1	1	no	1	4	No	Yes
ATN18	None	No	None	16	1	12	yes	1	11	Yes	Yes
ATN19	RIFLE-F	No	Diuretics, Other	24	3	6	no	3	7	No	Yes
ATN20	RIFLE-F	No	Contrast, Diuretics	3.5	0	22	yes	1	18	Yes	Yes
ATN21	None	No	Diuretics	18	1	11	Yes	1	6	No	No
ATN22	RIFLE-F	No	Aminoglycosides, Diuretics	20	1	14	Yes	2	18	No	No
ATN23	RIFLE-F	Yes	Contrast	42	4	15	no	3	16	No	Yes
ATN24	RIFLE-F	Yes	Amphotericin, Diuretics	23	2	8	Yes	3	10	No	No
ATN25	RIFLE-F	Yes	Amphotericin	12	0	5	yes	1	8	No	No
ATN26	RIFLE-F	Yes	Contrast, Rhabdomyolysis, Diuretics	48	2	7	yes	3	12	No	No
ATN27	RIFLE-F	Yes	Diuretics	38	3	3	no	3	1	No	Yes
ATN28	None	No	Contrast	27	3	5	No	2	3	No	Yes
ATN29	RIFLE-F	Yes	Diuretics	45	2	11	Yes	2	9	No	No
ATN30	RIFLE-F	Yes	Diuretics	12	0	19	No	1	18	No	Yes
ATN31	RIFLE-F	Yes	Contrast	43	3	10	No	3	7	No	Yes
ATN32	None	No	Diuretics, Starch	36	4	9	Yes	3	4	No	No

ATN33	None	No	None	9	0	2	No	1	5	No	Yes
ATN34	RIFLE-F	Yes	Contrast	98	0	11	Yes	2	9	No	No

AKI= acute kidney injury; RIFLE=Risk, Injury, Failure, Loss and End stage kidney injury score for AKI;
ATN=acute tubular necrosis; SQS=Semi-Quantitative Score.