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Original Article

ORIGINAL ARTICLE

Critically ill patients with acute kidney injury: clinical determinants and post-mortem histology

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ABSTRACT

Background. Acute kidney injury (AKI) requiring renal replacement therapy (RRT) in the intensive care unit (ICU) portends a poor prognosis. We aimed to better characterize predictors of survival and the mechanism of kidney failure in these patients.

Methods. This was a retrospective observational study using clinical and radiological electronic health records, analysed by univariable and multivariable binary logistic regression. Histopathological examination of post-mortem renal tissue was performed.

Results. Among 157 patients with AKI requiring RRT, higher serum creatinine at RRT initiation associated with increased ICU survival [odds ratio (OR) 0.33, 95% confidence interval (CI) 0.17–0.62, $P = .001$]; however, muscle mass (a marker of frailty) interacted with creatinine ($P = .02$) and superseded creatinine as a predictor of survival (OR 0.26, 95% CI 0.08–0.82; $P = .02$). Achieving lower cumulative fluid balance (mL/kg) predicted ICU survival (OR 1.01, 95% CI 1.00–1.01, $P < .001$), as supported by sensitivity analyses showing improved ICU survival with the use of furosemide (OR 0.40, 95% CI 0.18–0.87, $P = .02$) and increasing net ultrafiltration (OR 0.97, 95% CI 0.95–0.99, $P = .02$). A urine output of >500 mL/24 h strongly predicted successful liberation from RRT (OR 0.125, 95% CI 0.05–0.35, $P < .001$). Post-mortem reports were available for 32 patients; clinically unrecognized renal findings were described in 6 patients, 1 of whom had interstitial nephritis. Experimental staining of renal tissue from patients with sepsis-associated AKI (S-AKI) showed glomerular loss of synaptopodin ($P = .02$).

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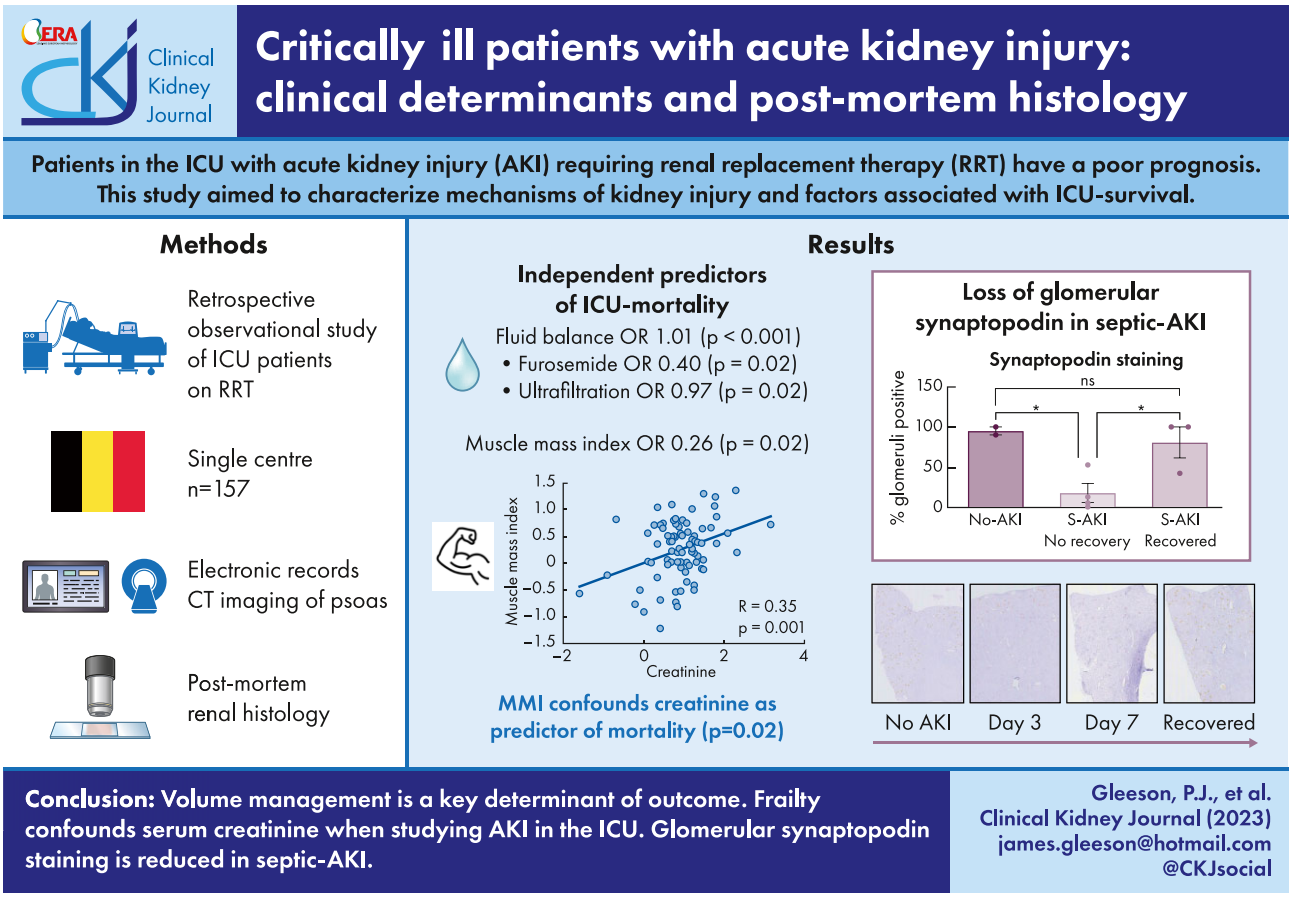
Conclusions. Confounding of creatinine by muscle mass undermines its use as a marker of AKI severity in clinical studies. Volume management and urine output are key determinants of outcome. Loss of synaptopodin implicates glomerular injury in the pathogenesis of S-AKI.

GRAPHICAL ABSTRACT



Keywords: AKI, creatinine, histology, muscle mass, sepsis

INTRODUCTION

Acute kidney injury (AKI) requiring renal replacement therapy (RRT) affects about one in eight critically ill patients [1] and carries a poor prognosis [2, 3]. Implementation strategies for RRT are evolving to optimize timing of initiation, volume management and timing of withdrawal [4]. The underlying mechanisms of renal failure in critically ill patients have not been fully elucidated [4].

Five recent trials have investigated when to start RRT in AKI—study designs varied and the results have been difficult to reconcile [5–9]. The role of ultrafiltration or loop diuretics in individualized volume management has yet to be defined [10]. Avoiding unnecessary RRT is desirable as it is resource intensive, venous catheters carry risk and beneficial substances are removed causing 'dialytrauma' [11]. Currently, the appropriate time to stop RRT is left to clinical judgement [12]. The underlying cause of AKI in critically ill patients is often undetermined, as renal biopsy is rarely considered appropriate in this setting [4].

An aim of this retrospective observational study was to identify factors associated with intensive care unit (ICU) mortality and renal recovery among critically ill patients requiring RRT. As serum creatinine is derived from skeletal muscle, computed tomography (CT) imaging was used to analyse the relationship between muscle mass and serum creatinine in AKI. Post-mortem reports available from non-survivors were used to characterize the underlying causes of AKI. Albuminuria is found in sepsis-associated AKI (S-AKI) [13], leading us to investigate a glomerular defect in S-AKI using immunohistochemistry of post-mortem renal tissue.

MATERIALS AND METHODS

Study population

All patients requiring continuous RRT (CRRT) at Hôpital Erasme Department of Intensive Care, Brussels, Belgium between January 2012 and December 2013 were identified retrospectively using the department registry. Patients were excluded if they were

under 18 years old, dialysis-dependent prior to admission, on CRRT for removal of poisons without renal failure, transferred to another hospital while on CRRT or died within 24 h of ICU admission. Ethical approval for this retrospective use of data and post-mortem tissue was granted by the local committee (P2013/232).

Data collection and definitions

Electronic medical records were reviewed for clinical details, time of initiation and ending of CRRT, fluid administration, ultrafiltration rate, urine output, patient weight, drug administration, and blood and urine biochemistry. Enteral feeding and faecal losses were not included in the calculation of fluid balance.

Residual creatinine clearance (mL/min) during the last 24 h of RRT was calculated using a spot urinary creatinine concentration ($U_{[creat]}$) at the end of the 24 h, 24-h urine volume (V) and the average serum creatinine concentration from the beginning and end of the 24-h period $[(S_{[creat]0} + S_{[creat]24})/2]$, with the formula $(U_{[creat]} \times V)/\{[(S_{[creat]0} + S_{[creat]24})/2] \times 1440\}$.

Muscle mass index

We analysed available CT images incorporating the lumbar spine taken between 1 year pre- and 14 days post-initiation of CRRT. Psoas muscle surface area was measured on axial sections at the level of mid-L3 [14] (Supplementary data, Fig. S1). The vertical height of the L3 vertebral body was measured on coronal sections and used as a proxy marker for height, as validated by Klein *et al.* [15]. Muscle mass index (MMI) was calculated as [Psoas Muscle Surface Area (cm^2)]/[L3 Height squared (cm squared)] [2].

Histology and immunohistochemistry

To investigate whether podocytes de-differentiate towards an immune phenotype [16] in sepsis, immunohistochemistry for CD80, a co-stimulatory molecule, was performed. Tissue was also stained for synaptopodin.

Formalin-fixed, paraffin-embedded tissue sections mounted on Superfrost slides were de-paraffinized and rehydrated, then incubated for 36 min (synaptopodin) or 52 min (CD80) at 100°C in EDTA buffer. Tissue was then incubated with the mouse monoclonal anti-CD80 antibody for 8 h (1:100, R&D Systems) or with the mouse monoclonal anti-synaptopodin antibody for 1 h (1:100, Progen). For CD80, slides were incubated with a biotinylated secondary antibody while for synaptopodin, slides were washed and incubated with discovery OmniMap anti-mouse HRP for 16 min. Slides were counterstained with haematoxylin.

Criteria defined by Kocovski *et al.* were used to determine whether histological changes were due to ante-mortem tubular injury or post-mortem autolysis based on the presence of tubular epithelial whorls, tubulorrhexis and interstitial expansion [17] (Supplementary data, Fig. S2). Slides were reviewed by an independent renal pathologist blinded to clinical details (A.S.).

Statistics

Univariable and multivariable binary logistic regression was performed to identify factors significantly associated with death prior to discharge from the ICU (ICU mortality) and independence from renal replacement therapy after stopping initial CVVH (renal recovery). Multivariable models were adjusted for gender and APACHE II score with other variables included as indicated in table legends. Volumes including net fluid balance, ultrafiltration and urine output were normalized to patients' weight (kg). Variables with a non-Gaussian distribution were transformed using their natural log (ln). Multiple collinearity was tested for (Variance Inflation Factor cut-off = 5) and eliminated using centred variables. ANOVA was used to compare histological findings using Prism (Graphpad Software, San Diego, CA, USA). Binary logistic regression analyses were performed using SPSS (IBM, New York, NY).

RESULTS

Study population

During the 2-year study period, 291 patients required CRRT and 157 met inclusion criteria. The main reason for exclusion was death within 24 h of commencing RRT (Fig. 1). The mean [interquartile range (IQR)] age was 63 (54–72) years and 96 (61%) patients were male. The median (IQR) lactate on ICU admission was 2.6 (1.5–5.4) mMol and the median (IQR) APACHE II score was 23 (17–26). Twenty-four patients (15%) received concurrent extra-corporeal membrane oxygenation (ECMO) support. Eighty patients (51%) died during their ICU admission (Table 1).

Sixty-six patients (42%) had a history of hypertension and 53 (34%) had pre-existing chronic kidney disease (CKD). In univariable analyses, patients with arterial hypertension (OR 0.5, 95% CI 0.26–0.95, $P = .03$) or CKD (OR 0.5, 95% CI 0.26–0.98, $P = .04$) at baseline were less likely to die in the ICU, so these variables were included in all multivariable models.

Sixty-seven patients (42.7%) had a diagnosis of septic shock and 40 patients (25.5%) had a diagnosis of cardiogenic shock, which both predicted ICU mortality in univariable and multivariable analyses. Acute respiratory distress syndrome (ARDS) predicted ICU mortality in univariable analysis. Septic shock, cardiogenic shock and ARDS were included in multivariable models of ICU mortality for the full cohort (Tables 1 and 2).

Clinical and post-mortem diagnoses for AKI

Clinically, the cause of AKI was attributed to acute tubular injury (ATI) in 77% ($n = 121$) of cases, cardiorenal syndrome in 8% ($n = 12$), other intrinsic renal causes in 6% ($n = 10$) and hepatorenal syndrome in 5% ($n = 8$); there was one case of obstructive nephropathy. Renal insufficiency was felt to be functional (prerenal azotemia) in five cases (3%) (Fig. 2).

Post-mortem reports were available for renal tissue from 32 patients. Four samples were reported as being uninterpretable due to post-mortem artefact. Clinically unrecognized diseases were diagnosed by post-mortem examination in six patients: three had chronic pyelonephritis, one had bilateral renal infarction, one had septic emboli and one had interstitial nephritis.

CRRT: initiation

Median (IQR) time from ICU admission to starting CRRT was 2 days (1–4). Chronological time from ICU admission to starting CRRT was not significantly associated with ICU mortality in univariable (OR 1.1, 95% CI 0.99–1.20, $P = .07$) or multivariable (OR 1.00, 95% CI 0.88–1.14, $P = .98$) analyses. Urea and creatinine median levels (IQR) at the start of CRRT were 97 mg/dL (65–150) and 2.6 mg/dL (1.9–3.9), respectively. Higher urea concentration at the start of CRRT was a significant predictor of ICU survival in univariable analysis (OR 0.58, 95% CI 0.35–0.97, $P = .04$) but lost significance in multivariable analysis (OR 0.60, 95% CI 0.32–1.11, $P = .1$) and remained insignificant after adjusting for

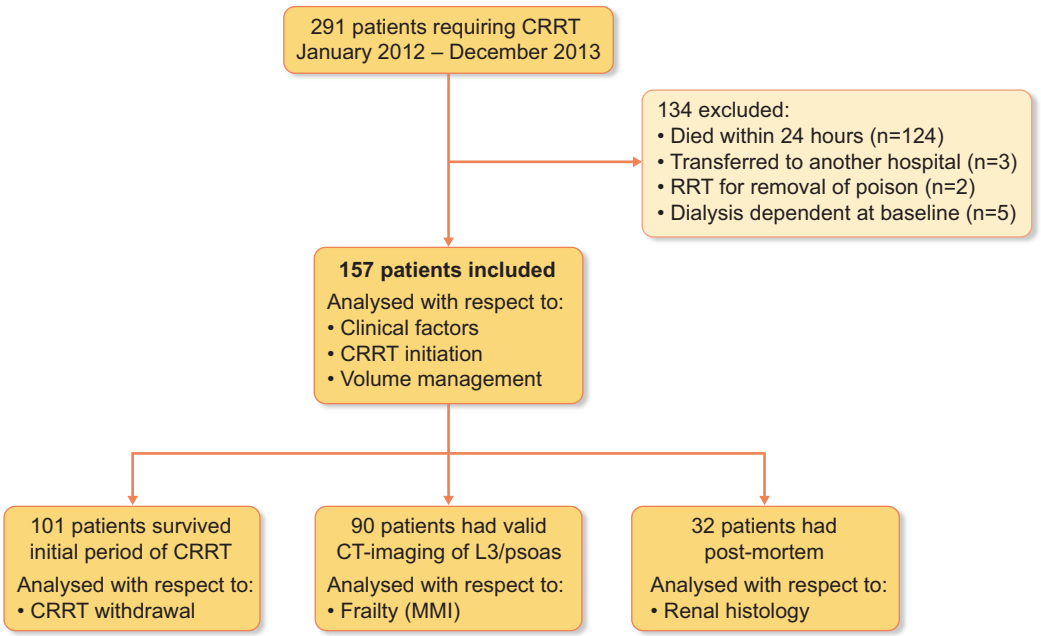


Figure 1: Flowchart of patient inclusion and exclusion for different aspects of this retrospective observational study.

Table 1: Patient characteristics.

Baseline demographics and characteristics ($n = 157$)	Total population ($n = 157$)	ICU survivors ($n = 77$)	ICU non-survivors ($n = 80$)	P-value[a] survivors vs non-survivors
Age, years [median (IQR)]	63 (54–72)	63 (56–72.5)	62.5 (50.3–71.5)	.42
Male [*n* (%)]	96 (61)	45 (58)	51 (64)	.50
Weight, kg [mean (SD)]	78.1 (19.7)	78 (60–90)	80 (60–90)	.39
Hypertension [*n* (%)]	66 (42)	39 (51)	27 (34)	.03
CKD [*n* (%)]	53 (33.8)	32 (42)	21 (26.3)	.04
ICU clinical characteristics				
Admission lactate, mMol [median (IQR)]	2.6 (1.5–5.4)	2.4 (1.2–5.0)	3.1 (1.7–6.1)	.05
APACHE II score [mean (SD)]	22.1 (7.6)	23 (16.5–26.0)	22 (17–27.8)	.58
Septic shock [*n* (%)]	67 (42.7)	24 (31)	43 (54)	.004
Cardiogenic shock [*n* (%)]	40 (25.5)	13 (17)	27 (34)	.02
Decompensated cirrhosis [*n* (%)]	26 (16.6)	12 (16)	14 (17.5)	.75
GI bleeding [*n* (%)]	13 (8.3)	5 (6.5)	8 (10)	.44
ECMO [*n* (%)]	24 (15.3)	5 (6.5)	19 (24)	.003
CRRT-related characteristics				
Chronological time to start CRRT, days [median (IQR)]	2 (1–4)	2 (1–3)	2 (1–4.8)	.12
Creatinine at start, mg/dL [median (IQR)]	2.6 (1.9–3.9)	3.1 (2.05–5.35)	2.2 (1.6–3.3)	<.001
Urea at start, mg/dL [median (IQR)]	97 (65–150)	100 (70.5–170)	93.5 (57.8–140)	.07
Duration of CRRT, days [median (IQR)]	6 (3–9)	6 (3–9)	6 (4–11)	.18
Ultrafiltration per kg per day, mL [mean (SD)]	21 (16.6)	23.2 (17.5)	18.8 (15.5)	.12
Cumulative net fluid balance during CRRT per kg, mL [median (IQR)]	43.3 (–49.5 to 151.3)	–6.3 (–85.4 to 56.1)	87.6 (26.2–193.3)	<.001
Urine output during last 24 h of CRRT, mL [median (IQR)]	200 (12.5–1100)	650 (153–1575)	40 (0–315)	<.001
Creatinine clearance over last 24 h of CRRT, mL/min [median (IQR)]	4.3 (0–21.5)	11.7 (1.3–25.8)	1 (0–15.2)	<.001
Loop diuretic use [*n* (%)]	78 (50)	42 (55)	35 (44)	.15
Cumulative furosemide dose ($n = 78$), mg [median (IQR)]	250 (120–500)	325 (125–589)	160 (80–410)	.16

[a]Chi-square test for comparison of proportions, *t*-test for comparison of means and Mann–Whitney test for comparison of medians.

Table 2: Predictors of ICU mortality.

	Univariable			Multivariable		
	OR	(95% CI)	P	OR	(95% CI)	P
ICU clinical factors[a]						
Septic shock	2.6	(1.3–4.9)	.005	2.33	(1.17–4.63)	.016
Cardiogenic shock	2.5	(1.2–5.3)	.017	2.94	(1.33–6.49)	.008
ARDS	2.6	(1.3–5.4)	.009	2.12	(0.98–4.58)	.056
Decompensated cirrhosis	1.1	(0.8–1.2)	.747	1.08	(0.43–2.67)	.877
Renal factors[b]						
Creatinine at CRRT start [ln(mg/dL)]	0.33	(0.19–0.59)	<.001	0.35	(0.18–0.70)	.003
Urea at CRRT start [ln(mg/dL)]	0.58	(0.35–0.97)	.04	0.60	(0.32–1.11)	.104
Urine output (mL/kg/h) at CRRT start	0.74	(0.57–0.95)	.02	0.75	(0.57–0.99)	.041
Net fluid balance during CRRT (mL/kg)	1.01	(1.00–1.01)	.001	1.01	(1.01–1.01)	<.001
Ultrafiltration rate (mL/kg/day)	0.984	(0.96–1.00)	.118	0.97	(0.95–0.99)	.02
Furosemide administration (yes versus no)	0.63	(0.34–1.19)	.151	0.40	(0.18–0.87)	.02

Binary logistic regression multivariable models were adjusted for the following covariables:
[a]gender, APACHE II (including age), hypertension and CKD.
[b]gender, APACHE II (including age), hypertension, CKD, chronological time to CRRT start, septic shock, cardiogenic shock and ARDS.

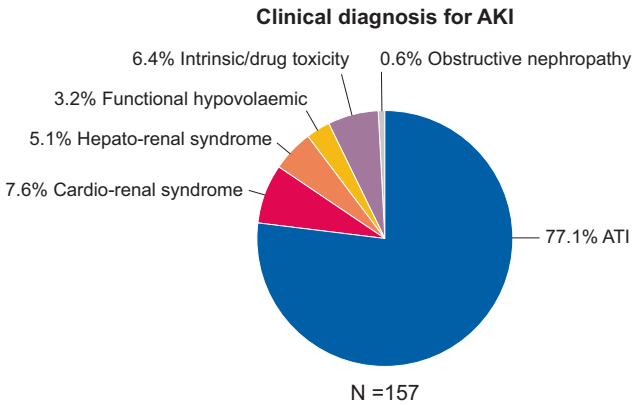


Figure 2: Proportions of clinical diagnoses attributed as the cause of AKI for all patients (n = 157) as determined by retrospective review of electronic medical records.

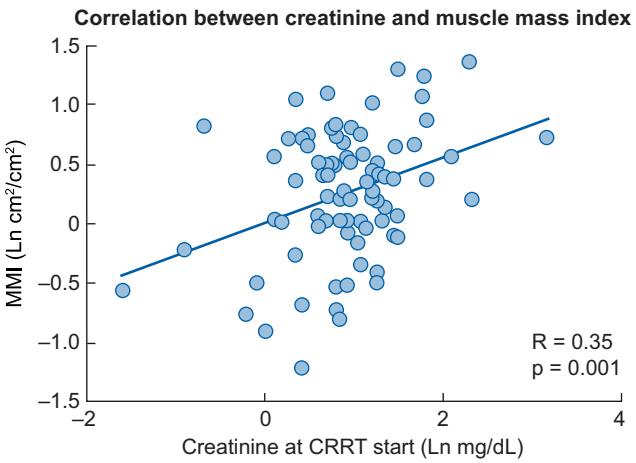


Figure 3: Correlation between serum creatinine level (mg/dL) at initiation of CRRT and muscle mass index (MMI) [psoas muscle area at the level of L3 (cm^2)/height of the L3 vertebral body squared (cm^2)] among patients with available CT imaging (n = 90). The natural log (Ln) for both sets of values was used as they had non-parametric distributions.

corticosteroids and gastrointestinal (GI) bleeding, which can confound blood urea levels. Higher serum creatinine at the time of starting CRRT was a strong predictor of ICU survival in both univariable (OR 0.33, 95% CI 0.19–0.59, $P < .001$) and multivariable analyses (OR 0.35, 95% CI 0.18–0.70, $P = .003$) (Table 2).

MMI confounds serum creatinine

To address potential confounding of serum creatinine levels by muscle mass, radiology records were reviewed and CT images capturing psoas muscles at the level of L3 were available for 90/157 patients (57%), allowing calculation of a MMI as described in Materials and methods, and Supplementary data, Fig. S1.

MMI correlated significantly with plasma creatinine at the start of CRRT (R = 0.35; $P = .001$) (Fig. 3). MMI and creatinine interacted significantly with respect to ICU mortality (OR 0.37, 95% CI 0.16–0.85, $P = .02$). When MMI is included in the model, with variable-centering to account for collinearity, creatinine lost its predictive power for ICU mortality (OR 0.72, 95% CI 0.32–1.65, $P = .44$) while MMI remained a significant predictor of ICU mortality (OR 0.26, 95% CI 0.08–0.82, $P = .02$). Psoas muscle size was seen to increase significantly in rhabdomyolysis (OR 6.76, 95% CI 1.3–35.1, $P = .02$) and this was adjusted for in the model (Table 3).

CRRT: volume management

Mean (±SD) daily net ultrafiltration volume for each patient was 21 mL/kg (16.6). Furosemide was given to 78 patients (58%) and the median (IQR) cumulative dose received during CRRT was 250 mg (120–500) per patient. The median (IQR) cumulative net fluid balance for each patient over the total period of CRRT was +43.3 mL/kg (–49.5 ± 151.3) (Table 1).

Greater negative balance during CRRT was associated with higher survival in both univariable (OR 1.01, 95% CI 1.00–1.01, $P < .001$) and multivariable analyses (OR 1.01, 95% CI 1.01–1.01, $P < .001$). In keeping with this, higher rates of net ultrafiltration (OR 0.97, 95% CI 0.95–0.99, $P = .02$) and administration of furosemide (OR 0.40, 95% CI 0.18–0.87, $P = .02$) were independent predictors of ICU survival (Table 2). Increasing cumulative dose

Table 3: Predictors of ICU mortality among patients with CT imaging of psoas muscles.

	OR	95% CI	P
Interaction effect			
MMI*StartCreat[a]	0.37	0.16–0.85	.019
Fully adjusted with main effects and interaction effect			
Start Creatinine[b]	0.72	0.32–1.65	.439
Muscle Mass Index[b]	0.26	0.08–0.82	.021
Interaction MMI[b]*StartCreat[b]	0.72	0.20–2.58	.613

[a]Univariable analysis; patients with rhabdomyolysis were excluded.
[b]Values were centered to reduce multi-collinearity.
Fully adjusted model included the following covariables: gender, APACHE II, hypertension, CKD, chronological time to CRRT start and rhabdomyolysis.
StartCreat, Start Creatinine.

Table 4: Predictors of renal recovery among patients surviving initial CRRT by multivariable logistic regression analysis.

	OR	95% CI	P
CKD[a]	2.79	1.11–7.02	.03
Ultrafiltration rate (mL/kg/day)[b]	1.04	1.01–1.08	.008
Net fluid balance during CRRT (mL/kg)[b]	1.00	0.99–1.01	.898
Receiving loop diuretic[b]	0.80	0.32–1.96	.623
Duration of CRRT (days)[b]	1.04	0.98–1.1	.191
Urine output over last 24 h of CRRT [ln(mL)][b]	0.49	0.35–0.68	<.001
Calculated creatinine clearance over last 24 h of CRRT [ln(mL/min)][b]	0.52	0.38–0.72	<.001

Binary logistic regression models were adjusted for the following co-variables.
[a]gender, APACHE II and hypertension.
[b]gender, APACHE II, hypertension and CKD.

of furosemide predicted ICU survival in multivariable analysis (ln(mg) OR 0.84, 95% CI 0.73–0.97, $P = .02$).

Renal recovery and stopping CRRT

Fifty-six patients (35.7%) died during their initial period of CRRT. Among patients who survived the initial period of CRRT ($n = 101$), median (IQR) CRRT duration was 6 days (3–9). Urine output during the last 24 h before stopping initial CRRT in this population was 200 mL (median; IQR 12.5–1100) and the calculated creatinine clearance was 4.3 mL/min (median; IQR 0–21.5). Thirty-six of these patients (36%) required further RRT during their ICU admission and 24 (24%) of them died. These patients were analysed separately using multivariable models to identify factors that associated with renal recovery or mortality.

The duration of CRRT did not predict need for further RRT (OR 1.05, 95% CI 0.99–1.12, $P = .10$). CKD at baseline was a predictor of having recourse to further RRT (intermittent haemodialysis or CRRT) after initially stopping CRRT (OR 2.79, 95% CI 1.11–7.02, $P = .03$) (Table 4).

While increased net ultrafiltration (mL/kg/day) predicted ICU survival, it also predicted a need to re-start RRT (OR 1.04, 95% CI 1.01–1.08, $P = .008$). Furosemide administration had no effect on renal recovery (OR 0.80, 95% CI 0.32–1.96, $P = .62$) (Table 4).

Urine output during the last 24 h of CRRT (OR 0.49, 95% CI 0.35–0.68, $P < .001$) and creatinine clearance during the last 24 h of CRRT (OR 0.52, 95% CI 0.38–0.72, $P < .001$) both predicted renal recovery. A urine output of at least 500 mL during the last 24 h of CRRT strongly predicted independence from further RRT (OR 0.125, 95% CI 0.05–0.35, $P < .001$), regardless of whether or not diuretics were used (Table 4).

In this subgroup of initial CVVH survivors, neither length of time on CRRT (OR 1.01, 95% CI 0.97–1.06, $P = .57$), urine output at the end of CRRT (OR 0.89, 95% CI 0.65–1.23, $P = .48$) nor creatinine clearance at the end of CRRT (OR 0.92, 95% CI 0.78–1.07, $P = .275$) predicted survival to discharge from the ICU.

Histopathology and immunohistochemistry in S-AKI

Renal tissue was available from seven patients who died after developing S-AKI and two control patients who died with an infection, but without AKI. Histological changes were determined to be ante-mortem in all cases (Supplementary data, Fig. S2). Changes of ATI were focal and heterogenous. In the early phase of S-AKI, tubular injury was disproportionately mild relative to the degree of renal failure and became more severe as time on CRRT before death increased (Table 5, Supplementary data, Fig. S3).

CD80 staining was positive on tissue-resident lymphocytes (internal control) but there was no glomerular CD80 staining from patients with or without S-AKI, nor in control nephrectomy tissue (Fig. 4A).

Glomerular synaptopodin staining was significantly reduced in patients with active S-AKI compared with controls and patients who had recovered renal function (ANOVA $P = .02$, Fig. 4B). Progressive loss of synaptopodin staining was seen in S-AKI, starting with juxta-medullary glomeruli and moving outward to sub-capsular glomeruli. Expression reduced proportionally with duration of AKI, and normalized in patients that recovered renal function (Fig. 4C, Table 5).

DISCUSSION

In this retrospective observational study we found that muscle mass is an important confounder of serum creatinine in AKI, furosemide could be a safe adjunct to volume management during CRRT and patients with a urine output of >500 mL/24 h in the ICU are likely to become independent from RRT. Glomerular

Table 5: Histopathological findings of patients with S-AKI.

	Light microscopy			Synaptopodin IHC		Clinical	
Patient No.	Whorls (0–3+)	Tubullorhexis (0–3+)	Interstitial expansion (0–3+)	% Glomeruli +	Intensity (0–3+)	Days on CRRT	Days between death and renal recovery
Infection without AKI							
1	0	0	0	100	3+		
2	0	0	0	90	3+		
Sepsis with AKI—no recovery							
7	1+	0.5+	0.5+	52.5	1.5+	3	
5	1.5+	2+	1.5+	12.5	1.5+	7	
10	2+	2+	2+	5	0.5+	7	
6	3+	3+	3+	0	0	11	
Sepsis with AKI—recovered							
4	n/a	n/a	n/a	42	1.5+	5	2
9	1+	1+	1.5+	100	3+	4	6
8	0.5+	0	1+	100	3+	13	6

Renal tissue available from seven patients who died after suffering S-AKI and two control patients who died with an infection, but without AKI, were analysed by a renal pathologist blinded to clinical details. Tissue stained by periodic acid–Schiff was used for light microscopy and scored on a scale of 0 to 3+ for the presence of whorls, tubullorhexis and interstitial expansion. Slides used for immunohistochemistry staining of synaptopodin were scored for the extent of glomerular synaptopodin staining (synaptopodin-positive glomeruli as a % of all glomeruli) and the intensity of staining on a score of 0 to 3+, with 3+ being the greatest intensity. Two sections were available for each patient, and the average scores are reported here for each patient.
IHC, immunohistochemistry; n/a = not available.

staining for synaptopodin—part of the podocyte cytoskeleton—was found to be reduced in S-AKI.

Our initial finding that higher creatinine at the time of starting RRT favours ICU survival has been reported from another observational study [3], but should not be interpreted to mean starting RRT late is beneficial. Serum creatinine, produced by skeletal muscle, has long been a measure of renal function and its imperfections are well recognized [18]. Models suggest that in AKI, the rise in creatinine is not linear and creatinine kinetics would be affected by muscle mass [19]. Reduced muscle mass on cross-sectional imaging is associated with poorer surgical and ICU outcomes [20]. Muscle mass is a determinant of frailty that can be measured using psoas muscle size normalized to height [14], and recent work has also identified frailty as an important predictor of outcome in critically ill patients [21].

It is not surprising, then, that we found a significant interaction between MMI and serum creatinine at the time of RRT initiation with respect to ICU survival. Importantly, MMI superseded creatinine as a predictor of ICU survival, suggesting that serum creatinine levels are confounded by frailty. However, frailty is a multi-faceted syndrome [22]. The MMI used here only addresses sarcopenia, and has not been validated as a marker of frailty. Future studies should clarify how cross-sectional muscle mass on CT imaging relates to global measures of frailty, serum creatinine and clinical outcomes in AKI.

Similarly, the association of higher urea level at time of CRRT initiation with ICU survival in univariable analysis could be explained by better baseline nutritional status [23], rather than any advantage of delaying initiation of CRRT. This signal was lost in multivariable analysis.

The ELAIN trial found that starting RRT for septic patients with KDIGO stage 2 was better than waiting until they reached stage 3 [5]. However, any benefit from an early strategy has been refuted by the artificial kidney initiation in kidney injury (AKIKI), initiating dialysis early and late (IDEAL), and standard versus accelerated initiation of renal-replacement therapy in acute kidney injury (STARRT-AKI) investigators [6, 8, 9]. On the other hand, AKIKI-2 warns against waiting too late [7]. A meta-analysis of available studies has found no difference in survival between 'early' and 'late' strategies, however there was a higher complication rate in those receiving 'early' RRT [24]. Based on supplementary data from the IDEAL and AKIKI studies, about 40% of patients were enrolled based on creatinine values alone. While the randomization process should preclude any bias, in light of our findings, confounding by frailty in these patients cannot be excluded without randomization stratified by frailty.

Murugan *et al.* have published two studies regarding the effect of net ultrafiltration rate on outcome, with conflicting results. In a secondary, observational, analysis of the RENAL trial they found that patients in the highest tertile of net ultrafiltration did worse than patients in the lowest tertile [25] while in another study they found that higher rates of ultrafiltration were associated with better survival [26]. Our findings concur with the latter study. However, sicker patients are less likely to tolerate fluid removal which, despite adjustment for illness severity, could confound our analysis.

Furosemide has been widely studied in AKI, and results have varied. A recent meta-analysis found that furosemide does not impact progression to RRT or mortality [27], and a large propensity-matched study of the MIMIC-III database found that furosemide administration promoted renal recovery and improved survival in AKI [28]. While furosemide clearly should not be given to hypovolaemic patients with AKI, its use as an adjunct to volume management in AKI with fluid overload is supported by guidelines [29]. Jeon *et al.* found that furosemide use was associated with successful discontinuation of CRRT and less time spent on CRRT [30]. We found that furosemide administration during CRRT was independently associated with ICU survival, without impacting renal recovery. A cumulative dose-dependent effect of furosemide on ICU survival could be explained by patients who respond to an initial dose being more likely to survive, and receive further doses. However, it is also consistent with a protective effect of greater net-negative fluid balance achieved using higher diuretic doses. Taken together, these results support a strategy of removing fluid when tolerated and using furosemide over ultrafiltration where possible.

Research has focused on timing of RRT initiation while timing of CRRT discontinuation remains under-investigated [10].

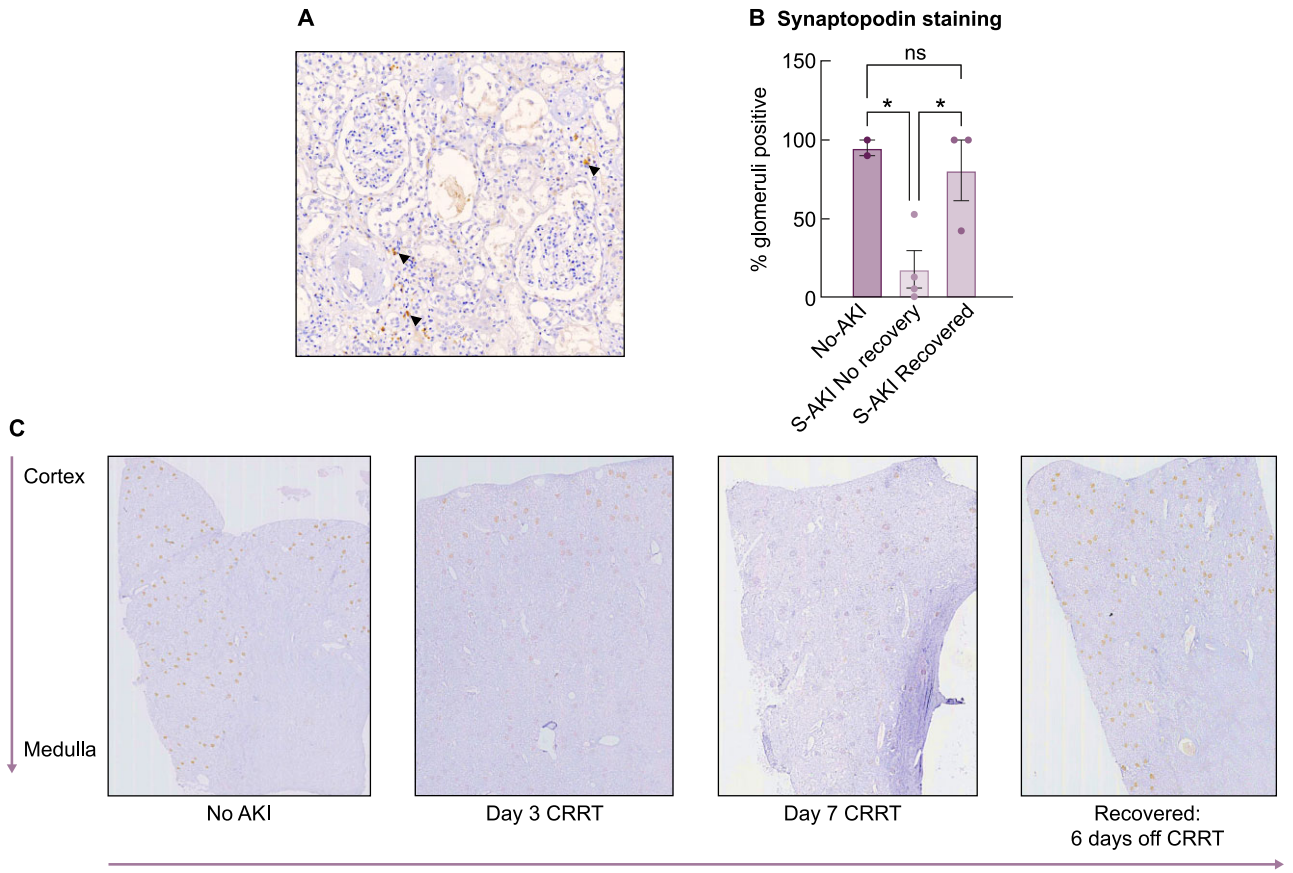


Figure 4: Light Microscopy and immunohistochemistry of tissue from patients with S-AKI and control patients with infection and no AKI. (A) CD80 IHC from a patient with S-AKI. Arrowheads show positive staining of resident interstitial antigen presenting cells. Glomerular staining is not greater than background. (B) Glomerular Synaptopodin staining in S-AKI (One-way ANOVA $P = .02$) (C) Representative synaptopodin staining of post-mortem renal tissue at different stages of S-AKI demonstrating juxta-medullary to cortical gradient loss of expression (magnification x5).

Urine output of >500 mL/24 h, regardless of diuretic exposure, strongly predicted successful CRRT discontinuation. An algorithm using urine output and furosemide responsiveness to guide CRRT discontinuation would be useful to investigate in future studies.

AKI is often considered to be a diagnosis but, actually, represents a syndrome with a myriad of different causes. This misconception hinders advancement in our understanding of the pathological processes driving AKI. Despite being a major cause of morbidity and mortality, the mechanism of S-AKI is not clear [4, 31]. Podocytes can behave as antigen-presenting cells, having phenotypic overlap with macrophages, and have been shown to cross-present antigens to T cells in a mouse model [16]. CD80 is an important co-stimulatory molecule for antigen presentation and is reportedly expressed by podocytes in other glomerular disease. Systemic administration of lipopolysaccharide upregulates podocyte CD80 expression in mice [32]. We sought to identify de-differentiation of podocytes to an immune phenotype in sepsis, however glomerular staining was negative for CD80 in S-AKI.

ATI was the most frequent clinical diagnosis made for AKI in this cohort. Tubular injury itself does not appear to explain the degree of renal dysfunction seen early in S-AKI, suggesting it is a proxy marker of another injurious process. Albuminuria is detected in S-AKI [13], suggesting glomerular injury. Synaptopodin is central to the podocyte cytoskeleton and its loss is associated with proteinuric kidney disease [33]. The reduction in glomerular synaptopodin staining in S-AKI seen here replicates findings from an animal model of sepsis [34]. The pattern of initial juxta-medullary loss of glomerular synaptopodin is interesting, as these glomeruli are gatekeepers of medullary blood-flow [35]. The arterioles of juxta-medullary glomeruli are of larger caliber and less affected by the vasoconstrictive effects of angiotensin II, in order to maintain downstream blood-flow to oxygen-sensitive tubules [36]. Synaptopodin loss could arise from direct inflammatory effects on glomeruli or result from shunting around these glomeruli, as has been described [35]. Podocytes support glomerular capillary tension [37] and it follows that decreased blood-flow through these juxta-medullary glomeruli would lead to downstream tubular injury. The small number of tissue samples available for immunohistochemistry in this study allow only for hypothesis generation—future studies should further explore loss of synaptopodin expression in S-AKI.

Unexpected acute interstitial nephritis was found in one patient—a diagnosis that could have changed management: withdrawing the offending drug and giving corticosteroids can speed up recovery [38]. Kidney biopsy is challenging in this population but, the clinico-pathological discordance rate of 19% seen with post-mortem tissue should be borne in mind.

Our study is limited by its retrospective, observational design and sample size. A significant proportion of ICU patients requiring CRRT who died within 24 h of admission were excluded, further limiting the generalizability of this single-centre study.

Post-mortem reports were not focused on renal tissue and artefactual changes could only be ruled out in a subset of tissue sections examined separately by a renal pathologist.

CRRT represents a significant perturbation of physiology that is resource intensive, mandates anticoagulation and requires dedicated venous catheters, while providing clearance of both noxious and beneficial substances. Inappropriate use of this intervention is unlikely to be inconsequential—defining optimal implementation strategies for CRRT remains a major challenge in nephrology and critical care medicine.

SUPPLEMENTRY DATA

Supplementary data are available at *ckj* online.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. This work has not been submitted for publication elsewhere.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

AUTHORS' CONTRIBUTIONS

P.J.G. gathered data, analysed data and wrote the first draft of the manuscript. I.A.C. gathered data and assisted with writing the manuscript. A.S. analysed post-mortem histopathological slides and assisted with preparing figures. C.K. and L.B. gathered and prepared histopathology slides. J.A. and S.R. performed experimental histopathological staining. D.J.S. assisted with data analysis. V.F. gathered data. J.C. was involved in clinical care and supervised the study. J.-L.V. and F.S.T. were involved in clinical care, supervised the study and were involved in preparation of the final manuscript.

REFERENCES

1. Hoste EA, Bagshaw SM, Bellomo R *et al*. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med* 2015;**41**:1411–23. https://doi.org/10.1007/s00134-015-3934-7
2. Rachoin JS, Weisberg LS. Renal replacement therapy in the ICU. *Crit Care Med* 2019;**47**:715–21. https://doi.org/10.1097/CCM.0000000000003701
3. Allegretti AS, Steele DJ, David-Kasdan JA *et al*. Continuous renal replacement therapy outcomes in acute kidney injury and end-stage renal disease: a cohort study. *Crit Care* 2013;**17**:R109. https://doi.org/10.1186/cc12780
4. Ronco C, Bellomo R, Kellum JA. Acute kidney injury. *Lancet North Am Ed* 2019;**394**:1949–64. https://doi.org/10.1016/S0140-6736(19)32563-2
5. Zarbock A, Kellum JA, Schmidt C *et al*. Effect of early vs delayed initiation of renal replacement therapy on mortality in critically ill patients with acute kidney injury: the ELAIN randomized clinical trial. *JAMA* 2016;**315**:2190–9. https://doi.org/10.1001/jama.2016.5828
6. Gaudry S, Hajage D, Schortgen F *et al*. Initiation strategies for renal-replacement therapy in the intensive care unit. *N Engl J Med* 2016;**375**:122–33. https://doi.org/10.1056/NEJMoa1603017
7. Gaudry S, Hajage D, Martin-Lefevre L *et al*. Comparison of two delayed strategies for renal replacement therapy initiation for severe acute kidney injury (AKIKI 2): a multicentre, open-label, randomised, controlled trial. *Lancet North Am Ed* 2021;**397**:1293–300. https://doi.org/10.1016/S0140-6736(21)00350-0
8. The STARRT-AKI Investigators for the Canadian Critical Care Trials Group, the Australian and New Zealand Intensive Care Society Clinical Trials Group, the United Kingdom Critical Care Research Group, the Canadian Nephrology Trials Network, and the Irish Critical Care Trials Group. Timing of initiation of renal-replacement therapy in acute kidney injury. *N Engl J Med* 2020;**383**:240–51. https://doi.org/10.1056/NEJMoa2000741
9. Barbar SD, Clere-Jehl R, Bourredjem A *et al*. Timing of renal-replacement therapy in patients with acute kidney injury and sepsis. *N Engl J Med* 2018;**379**:1431–42. https://doi.org/10.1056/NEJMoa1803213
10. Kelly YP, Waikar SS, Mendu ML. When to stop renal replacement therapy in anticipation of renal recovery in AKI: the need for consensus guidelines. *Semin Dial* 2019;**32**:205–9. https://doi.org/10.1111/sdi.12773
11. Maynar Moliner J, Honore PM, Sanchez-Izquierdo Riera JA *et al*. Handling continuous renal replacement therapy-related adverse effects in intensive care unit patients: the dialytrauma concept. *Blood Purif* 2012;**34**:177–85. https://doi.org/10.1159/000342064
12. Tandukar S, Palevsky PM. Continuous renal replacement therapy: who, when, why, and how. *Chest* 2019;**155**:626–38. https://doi.org/10.1016/j.chest.2018.09.004
13. Neyra JA, Manllo J, Li X *et al*. Association of de novo dipstick albuminuria with severe acute kidney injury in critically ill septic patients. *Nephron Clin Pract* 2014;**128**:373–80. https://doi.org/10.1159/000368902
14. Dahya V, Xiao J, Prado CM *et al*. Computed tomography-derived skeletal muscle index: a novel predictor of frailty and hospital length of stay after transcatheter aortic valve replacement. *Am Heart J* 2016;**182**:21–7. https://doi.org/10.1016/j.ahj.2016.08.016
15. Klein A, Nagel K, Guhrs J *et al*. On the relationship between stature and anthropometric measurements of lumbar vertebrae. *Sci Justice* 2015;**55**:383–7. https://doi.org/10.1016/j.scijus.2015.05.004
16. Goldwich A, Burkard M, Olke M *et al*. Podocytes are nonhematopoietic professional antigen-presenting cells. *J Am Soc Nephrol* 2013;**24**:906–16. https://doi.org/10.1681/ASN.2012020133
17. Kocovski L, Duflou J. Can renal acute tubular necrosis be differentiated from autolysis at autopsy? *J Forensic Sci* 2009;**54**:439–42. https://doi.org/10.1111/j.1556-4029.2008.00956.x
18. Schetz M, Schortgen F. Ten shortcomings of the current definition of AKI. *Intensive Care Med* 2017;**43**:911–3. https://doi.org/10.1007/s00134-017-4715-2
19. Waikar SS, Bonventre JV. Creatinine kinetics and the definition of acute kidney injury. *J Am Soc Nephrol* 2009;**20**:672–9. https://doi.org/10.1681/ASN.2008070669
20. Bentov I, Kaplan SJ, Pham TN *et al*. Frailty assessment: from clinical to radiological tools. *Br J Anaesth* 2019;**123**:37–50. https://doi.org/10.1016/j.bja.2019.03.034

21. Muscedere J, Waters B, Varambally A *et al*. The impact of frailty on intensive care unit outcomes: a systematic review and meta-analysis. *Intensive Care Med* 2017;**43**:1105–22. https://doi.org/10.1007/s00134-017-4867-0
22. De Biasio JC, Mittel AM, Mueller AL *et al*. Frailty in critical care medicine: a review. *Anesth Analg* 2020;**130**:1462–73. https://doi.org/10.1213/ANE.0000000000004665
23. Lee SW, Yang YM, Kim HY *et al*. Predialysis urea nitrogen is a nutritional marker of hemodialysis patients. *Chonnam Med J* 2022;**58**:69–74. https://doi.org/10.4068/cmj.2022.58.2.69
24. Castro I, Relvas M, Gameiro J *et al*. The impact of early versus late initiation of renal replacement therapy in critically ill patients with acute kidney injury on mortality and clinical outcomes: a meta-analysis. *Clin Kidney J* 2022;**15**:1932–45. https://doi.org/10.1093/ckj/sfac139
25. Murugan R, Kerti SJ, Chang CH *et al*. Association of net ultrafiltration rate with mortality among critically ill adults with acute kidney injury receiving continuous venovenous hemodiafiltration: a secondary analysis of the Randomized Evaluation of Normal vs Augmented Level (RENAL) of Renal Replacement Therapy Trial. *JAMA Netw Open* 2019;**2**:e195418.
26. Murugan R, Balakumar V, Kerti SJ *et al*. Net ultrafiltration intensity and mortality in critically ill patients with fluid overload. *Crit Care* 2018;**22**:223. https://doi.org/10.1186/s13054-018-2163-1
27. Krzych LJ, Czempik PF. Impact of furosemide on mortality and the requirement for renal replacement therapy in acute kidney injury: a systematic review and meta-analysis of randomised trials. *Ann Intensive Care* 2019;**9**:85. https://doi.org/10.1186/s13613-019-0557-0
28. Zhao GJ, Xu C, Ying JC *et al*. Association between furosemide administration and outcomes in critically ill patients with acute kidney injury. *Crit Care* 2020;**24**:75. https://doi.org/10.1186/s13054-020-2798-6
29. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract* 2012;**120**:c179–84. https://doi.org/10.1159/000339789
30. Jeon J, Kim DH, Baeg SI *et al*. Association between diuretics and successful discontinuation of continuous renal replacement therapy in critically ill patients with acute kidney injury. *Crit Care* 2018;**22**:255. https://doi.org/10.1186/s13054-018-2192-9
31. Lipcsey M, Bellomo R. Septic acute kidney injury: hemodynamic syndrome, inflammatory disorder, or both? *Crit Care* 2011;**15**:1008. https://doi.org/10.1186/cc10525
32. Reiser J, von Gersdorff G, Loos M *et al*. Induction of B7-1 in podocytes is associated with nephrotic syndrome. *J Clin Invest* 2004;**113**:1390–7. https://doi.org/10.1172/JCI20402
33. Perico L, Conti S, Benigni A *et al*. Podocyte-actin dynamics in health and disease. *Nat Rev Nephrol* 2016;**12**:692–710. https://doi.org/10.1038/nrneph.2016.127
34. Kato T, Mizuno-Horikawa Y, Mizuno S. Decreases in podocin, CD2-associated protein (CD2AP) and tensin2 may be involved in albuminuria during septic acute renal failure. *J Vet Med Sci* 2011;**73**:1579–84. https://doi.org/10.1292/jvms.11-0203
35. Calzavacca P, May CN, Bellomo R. Glomerular haemodynamics, the renal sympathetic nervous system and sepsis-induced acute kidney injury. *Nephrol Dial Transplant* 2014;**29**:2178–84. https://doi.org/10.1093/ndt/gfu052
36. Denton KM, Anderson WP, Sinniah R. Effects of angiotensin II on regional afferent and efferent arteriole dimensions and the glomerular pole. *Am J Physiol Regul Integr Comp Physiol* 2000;**279**:R629–38. https://doi.org/10.1152/ajpregu.2000.279.2.R629
37. Saleem MA, Zavadil J, Bailly M *et al*. The molecular and functional phenotype of glomerular podocytes reveals key features of contractile smooth muscle cells. *Am J Physiol Renal Physiol* 2008;**295**:F959–70. https://doi.org/10.1152/ajprenal.00559.2007
38. Clarkson MR, Giblin L, O'Connell FP *et al*. Acute interstitial nephritis: clinical features and response to corticosteroid therapy. *Nephrol Dial Transplant* 2004;**19**:2778–83. https://doi.org/10.1093/ndt/gfh485