Urine neutrophil gelatinase-associated lipocalin and urine output as predictors of the successful discontinuation of continuous renal replacement therapy in critically ill patients with acute kidney injury

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Abstract

Background: Continuous renal replacement therapy (CCRT) is a frequently used modality for the support of intensive care patients with acute kidney failure (AKI). Nevertheless, there are no objective criteria for the discontinuation of CRRT. The purpose of this study was to investigate whether urine neutrophil gelatinase-associated lipocalin (uNGAL) alone or in combination with urine output could be used as a diagnostic test for renal function recovery in ICU patients on CRRT.

Methods: This was a single-centre prospective observational study including patients with acute kidney failure needing CRRT. Sixty-nine patients were enrolled, and 54 completed the study. Of the 54 patients, 22 recovered renal function (REC), defined as dialysis independency at 72 hours from discontinuation, while 32 patients did not (NREC). Urine NGAL was measured at 0, 6, 12, and 24 hours after CRRT discontinuation. The cumulated urine output was measured for 24 hours prior to discontinuation and at 6, 12, and 24 hours after discontinuation. Logistic regression was used to calculate the missing 6-hour uNGAL values by interpolation. The Youden index was used to calculate cut-off values. A P-value <0.05 was considered statistically significant.

Results: Baseline characteristics at CRRT initiation were similar between groups. Compared to the NREC group, REC patients had significantly higher urine output ($p<0.0001$) and lower uNGAL ($p<0.001$) at all time points, except for uNGAL at 24 hours ($p<0.24$). The best single predictor for renal recovery was the cumulated urine output 24 hours after discontinuation (predictive value 83%). Combining uNGAL at 6 hours (cut-off 1650 µg/L) with the cumulated urine output during the 24 hours prior to discontinuation (cut-off 210 ml) proved superior, with predictive values of 92% (CRRT dependency) and 93% (renal function recovery).

Conclusions: With predictive values up to 93%, the combination of uNGAL at 6 hours and the cumulatated urine output during the 24 hours prior to CRRT cessation proved to be the best diagnostic test for renal function recovery in ICU patients.

Clinical trial registration: N/A

Background

Acute kidney injury (AKI) is a common complication in patients admitted to an intensive care unit (ICU). An international multicentre study found that 13.5% of patients admitted to the ICU required renal replacement therapy (RRT) [1]. The preferred choice of dialysis modality is continuous renal replacement therapy (CRRT), thereby obtaining better solute control and more stable haemodynamics. However, despite the frequent use of CRRT, there is no consensus regarding its discontinuation. Ideally, the physician would have access to a test with high diagnostic sensitivity for predicting renal recovery. This is, however, complicated by the complex physiology and individualized presentation of the critically ill patient. In clinical practice, CRRT is discontinued on an individual basis, i.e., when the physician assesses that the general state of the patient could indicate renal recovery. Urine output is currently the most valid

predictor for successful discontinuation [2–6]. Other less predictive variables are serum and urine creatinine, Sequential Organ Failure Assessment (SOFA) score, dialysis time, age, co-morbidities, and 2-hour creatinine clearance [2–7]. In recent years, specific biomarkers have been associated with renal injury and renal recovery. Currently, the most promising and thoroughly explored biomarker is neutrophil gelatinase-associated lipocalin (NGAL). NGAL has already been shown to be a very early predictor of AKI [8–13]. In cases in which the duration of trauma to the kidney is known, such as following organ transplantation and cardiac surgery, NGAL has shown excellent results. The landmark trial by Mishra and colleagues [11] from 2005 showed that urine NGAL (uNGAL) 2 hours after cardiopulmonary bypass had an area under the receiver operating curve (ROC) of 99.8% for the prediction of AKI. In the same study, the serum creatinine increased with a 1–3 day delay. Urine NGAL is an expression of ongoing *damage* to the kidney, whereas creatinine reflects the *function* of the kidney. A decrease in uNGAL might represent renal recovery. Few studies have focused on the ability of uNGAL to predict renal recovery in critically ill patients at the time of discontinuation of dialysis [14–16]. In these studies, uNGAL was determined 24 hours or later after discontinuation. It may be argued that the longer the timeframe from discontinuation until the uNGAL level is determined, the less clinically relevant the biomarker becomes.

The objectives of the present study were to investigate whether uNGAL as a diagnostic test determined 0–24 hours after discontinuation of CRRT is able to predict renal recovery in critically ill patients and whether a diagnostic test combining uNGAL with urine output could be an even better predictor of renal recovery.

Methods

Setting

This single-centre prospective observational study was conducted in the tertiary ICU at Odense University Hospital between May 2016 and April 2018. Patients with AKI, according to the RIFLE criteria, who were admitted to the ICU were included at the start of CRRT. The exclusion criteria were age < 18 years, chronic kidney failure and missing consent. Patient follow-up was 3 months after discharge from the ICU.

Continuous veno-venous haemodialysis (CVVHD) was the preferred renal replacement modality with multi-filtrate CiCa dialysate (Fresenius Medical Care, Bad Homburg, Germany). If the patient was otherwise ready to be transferred to the ward, haemodialysis was used instead of CRRT.

Endpoint

The successful endpoint with regard to the diagnostic test of uNGAL and urine output was defined as 3 dialysis-free days following the discontinuation of CRRT.

Data collection

Demographic and basic clinical information together with a baseline urine sample for uNGAL analysis were obtained at study inclusion.

At the time of the first cessation of CRRT, the following physiological and laboratory variables were collected: uNGAL at the time of cessation (0 hours) and at 6, 12, and 24 hours thereafter; mean arterial pressure at 0, 6, 12 and 24 hours; use of diuretics at 6, 12, and 24 hours (note that in our ICU unit, diuretics are not given during CRRT treatment); serum creatinine (s-Cr), creatinine clearance, and CRP at 0 and 24 hours; cumulated urine output for 24 hours prior to CRRT cessation (UOC24pre) and 24 hours after CRRT cessation (UOC24post); average hourly urine output during the first 6 hours (UOA6) and during the first 12 hours (UOA12) after cessation; and the time from CRRT cessation until the re-initiation of CRRT.

Outcome data

The outcomes were dialysis dependence and mortality at 3 months after ICU discharge.

Urine NGAL collection and analysis

Urine specimens for analysing uNGAL were collected primarily from the urinary catheter and, if that was not possible, from the urinary catheter bag. The urine sample was immediately transferred to a -80 °C freezer.

Urine NGAL was measured using an automated, turbidimetric immunoassay (NGAL Test Reagent Kit, BioPorto Diagnostics, Denmark) on a Roche Cobas 8000 analyser (Roche Diagnostics, Rotkreutz, Switzerland). The reference range for uNGAL (47–141 µg/L) was based on uNGAL measurements in 60 healthy adults aged 18–67 years using the same uNGAL method employed in the study.

Data processing and analysis

Study data were managed using REDCap (REDCap Consortium, Vanderbilt University Medical Center, Nashville, TN) electronic data capture tools in the Odense Patient Data Explorative Network (OPEN), hosted by the University of Southern Denmark and Odense University Hospital. All data were transferred to Excel 16 (Microsoft, Redmond, WA) for Mac and analysed using JMP 14.1 (SAS Institute, Cary, North Carolina) statistical software.

To validate the diagnostic tests for the successful discontinuation of CRRT, the patients were divided into the “recovery” (REC) and “non-recovery, all” (NREC-A) groups. Successful recovery was defined as the patient being independent from dialysis for at least 72 hours. The NREC-A group was subdivided according to whether the patient initiated haemodialysis (NREC-H) or reinitiated CRRT (NREC-C). The patients were excluded from analysis if they had died before discontinuation or if active treatment of the patient’s basic condition had been discontinued while they were on CRRT. Each patient was included only once.

Demographic variables and variables at discontinuation were compared between the REC and NREC-A groups using Student’s t-tests and Wilcoxon non-parametric tests for continuous variables. Fisher’s exact tests were used for categorical variables. $p < 0.05$ was considered statistically significant.

The NGAL values were shown to be log-normally distributed. Linear regression analysis of the correlations between log uNGAL at 6 hours and log uNGAL at 12 hours and between log uNGAL 6 at hours and log uNGAL at 0 hours was used to calculate the missing log uNGAL 6-hour values by interpolation.

All demographic variables and laboratory and clinical variables at discontinuation were analysed by multiple regression using a manual backward stepwise method to identify variables indicating the successful discontinuation of CRRT.

For all uNGAL (at 0, 6, 12 and 24 hours) and urine output (UOC24pre, UOC24post, OUA6 and OUA12) measures, the area under the receiver operating characteristic (ROC) curve, the negative predictive value (NPV), and the positive ("positive" outcome defined as the patient being dialysis dependent) predictive value (PPV) were calculated using Excel 16 (Microsoft, Redmond, WA) and Prism 8 (GraphPad, San Diego, CA). Youden indices (defined as NPV + PPV) were calculated to determine the cut-off values yielding the optimum combined NPV and PPV. Each of the identified uNGAL and urine output optimum cut-off values were permutated in "and" and "or" expressions to identify the combination of the 2 variables yielding the highest NPV [17], i.e., a "test" based on uNGAL and urine output, if negative, giving the most reliable prediction of renal function recovery.

Results

Sixty-nine patients were enrolled in the study. A total of 262 patients were treated with CRRT at the Odense University Hospital during the 2-year study period. A total of 193 (74%) patients were excluded, either because they did not meet the inclusion criteria or because of failure to collect urine within the inclusion window. In addition, 15 out of the 69 (22%) patients died while on CRRT. Of the remaining 54 patients, 22 (41%) experienced renal function recovery (REC), whereas 32 (59%) patients did not recover renal function (NREC-A). This NREC-A group of 32 was further subdivided into the NREC-C (20 [63%] patients) and NREC-H (12 [37%] patients) subgroups (Fig. 1). One patient in the REC group was placed back on CRRT after 92 hours of dialysis independence.

Table 1
Baseline characteristics at continuous renal replacement therapy initiation.

Clinical variables	Recovery (n = 22)	Non-recovery; CRRT re-initiation (n = 20)	Non-recovery; Haemodialysis initiation (n = 12)	P-value
Male/female	16/6 (73%/27%)	11/9 (55%/45%)	9/3 (75%/25%)	0.56
Age, years	77 [42–83]	70 [51–81]	68 [30–83]	0.84
BMI, kg/m^2	26 [19–35]	27 [19–38]	25 [24–37]	0.45
SOFA, highest value	13 [7–18]	15 [11–19]	13 [8–21]	0,20
APACHE II	27 [18–40]	29 [21–43]	26 [18–38]	0.71
SAPS II	56 [30–75]	65 [44–86]	58 [38–78]	0.10
Hypertension	9 (41%)	11 (55%)	7 (58%)	0.76
Malignity	6 (27%)	10 (50%)	2 (17%)	0.56
CVVH/CVVHD	0/22 (0/100%)	4/16 (20/80%)	0/12 (0/100%)	0.14
RIFLE-F	21 (95%)[a]	20 (100%)	12 (100%)	0.41
Furosemide, mg/day	0 [0-554]	80 [0-534]	100 [0-480]	0.44[d]
Mean arterial pressure, mmHg	69 [62–85]	70 [63–93]	78 [59–113]	0.23
Creatinine, µmol/L	186 [84–621]	194 [87–531]	238 [90–765]	0.99
Creatinine clearance, ml/min	19 [7–45]	15 [8–27]	17 [5–26]	0.11
C-Reactive Protein (CRP), mg/L	216 [7-454]	139 [13–266]	108 [34–423]	0,18
Urine output, ml/hour [b]	18 [0–50]	8 [0–97]	0 [0–52]	0.40[d]

P-value was calculated between the recovery and non-recovery (CRRT-re-initiation and haemodialysis) groups. CRRT, continuous renal replacement therapy; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation II; CVVH, continuous veno-venous haemofiltration; CVVHD, continuous veno-venous haemodialysis; AKI, acute kidney injury; NGAL, neutrophil gelatinase-associated lipocalin. Categorical variables are expressed as numbers (percentages). Continuous variables are expressed as medians [10%-90% quantiles]

a) RIFLE-I was recorded for one patient. b) Mean urine output 6 hours before dialysis initiation; if urine output < 5 ml in one hour, then it was recorded as 0 ml. c) Urine output 24 hours before CRRT initiation. d) Log10 transformation was used to calculate the p-value.

Clinical variables	Recovery (n = 22)	Non-recovery; CRRT re-initiation (n = 20)	Non-recovery; Haemodialysis initiation (n = 12)	P-value
Urine output, ml/day [c]	398 [103–1488]	326 [8-1750]	120 [13-2550]	0.23[d]
Noradrenaline, µg/kg/min	0,35 [0,09 – 1,26]	0,28 [0,07 – 1,32]	0,12 [0,02 – 1,10]	0.35[d]
Nephrotoxic medicine including contrast.	8 (36%)	12 (60%)	6 (50%)	0.18
Presumed primary cause of AKI: Prerenal Sepsis Glomerulonephritis Rhabdomyolysis Microthrombosis/ vasculitis	8 (36%) 12 (55%) 0 1 (4,5%) 1 (4,5%)	5 (25%) 13 (65%) 0 1 (5%) 1 (5%)	2 (17%) 4 (33%) 1 (8%) 3 (25%) 2 (17%)	0.56
Primary reason for ICU admission: Septic shock/sepsis Trauma AKI Respiratory failure Low cardiac output	9 (41%) 1 (5%) 5 (23%) 4 (18%) 3 (14%)	103(65%) 2 (10%) 1 (5%) 2 (10%) 2 (10%)	6 (51%) 0 2 (17%) 3 (34%) 1 (8%)	0.62
Mechanical ventilation, days	4 [0–23]	14[0–32]	2 [0–18]	0.18
ICU, days	10 [2–32]	17 [5–38]	9 [3–20]	0.22

P-value was calculated between the recovery and non-recovery (CRRT-re-initiation and haemodialysis) groups. CRRT, continuous renal replacement therapy; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation II; CVVH, continuous veno-venous haemofiltration; CVVHD, continuous veno-venous haemodialysis; AKI, acute kidney injury; NGAL, neutrophil gelatinase-associated lipocalin. Categorical variables are expressed as numbers (percentages). Continuous variables are expressed as medians [10%-90% quantiles]

a) RIFLE-I was recorded for one patient. b) Mean urine output 6 hours before dialysis initiation; if urine output < 5 ml in one hour, then it was recorded as 0 ml. c) Urine output 24 hours before CRRT initiation. d) Log10 transformation was used to calculate the p-value.

Clinical variables	Recovery (n = 22)	Non-recovery; CRRT re-initiation (n = 20)	Non-recovery; Haemodialysis initiation (n = 12)	P-value
Urine-NGAL, µg/L	2645 [279-27543]	2894 [749-18537]	3619 [128-30170]	0.57[d]

P-value was calculated between the recovery and non-recovery (CRRT-re-initiation and haemodialysis) groups. CRRT, continuous renal replacement therapy; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation II; CVVH, continuous veno-venous haemofiltration; CVVHD, continuous veno-venous haemodialysis; AKI, acute kidney injury; NGAL, neutrophil gelatinase-associated lipocalin. Categorical variables are expressed as numbers (percentages). Continuous variables are expressed as medians [10%-90% quantiles]

a) RIFLE-I was recorded for one patient. b) Mean urine output 6 hours before dialysis initiation; if urine output < 5 ml in one hour, then it was recorded as 0 ml. c) Urine output 24 hours before CRRT initiation. d) Log10 transformation was used to calculate the p-value.

Table 2
Variables at the time of discontinuation of continuous renal replacement therapy.

Variable	Recovery (n = 22)	Non-recovery; CRRT re-initiation (n = 20)	Non-recovery; Haemodialysis initiation (n = 12)	p-value
Urine NGAL	1370 [154–8002]	8934 [2095–40000]	8726 [360-35449]	0.0003[a]
time 0, µg/L	465 [56-3978]	2966 [1985–29011]	2854 [120–6055]	0.0006[a]
6 hrs, µg/L	436 [95-3780]	1976 [798-16972]	2148 [54-3825]	0.0008[a]
12 hrs, µg/L	455 [100–3337]	2290 [874-17665]	295 [32-1624]	0.24[a]
24 hrs, µg/L				
MAP	80 [67–103]	71 [62–94]	82 [67–96]	0.12
time 0, mmHg	78 [66–105]	72 [66–105]	78 [64–106]	0.30[a]
6 hrs, mmHg	75 [67–106]	73 [65–105]	80 [68–103]	0.42
12 hrs, mmHg	75 [67–106]	74 [67–100]	83 [68–113]	0.60
24 hrs, mmHg				
Furosemide	70 [0-240]	70 [0-246]	10 [0-240]	0.94
6 hrs., mg	95 [0-480]	100 [0-480]	130 [0-480]	0.66
12 hrs., mg	150 [0-853]	240 [15–936]	140 [0-944]	0.50
24 hrs., mg				
Use of other diuretics	4 (18%)	4 (20%)	6 (50%)	0.52
Urine output 24 hrs prior to CRRT discontinuation, ml	500 [87-2140]	100 [31–533]	20 [0-575]	< 0.0001
Urine output after discontinuation	65 [13–266]	8 [0–47]	3 [0–50]	< 0.0001
6 hrs, ml/hr	85 [27–197]	10 [0–60]	3 [0–54]	< 0.0001
12 hrs, ml/hr	2340 [828–4488]	480 [0-2952]	240 [0-3828]	< 0.0001
24 hrs, ml				
Time to re-initiation of CRRT, hrs		24 [6–64]		

Variable	Recovery (n = 22)	Non-recovery; CRRT re-initiation (n = 20)	Non-recovery; Haemodialysis initiation (n = 12)	p-value
Creatinine	98 [51–250]	130 [67–289]	157 [82–314]	0.18
At discontinuation, μmol/L	134 [67–340]	191 [119–409]	235 [135–433]	0.03
24 hrs after discontinuation, μmol/L				
Creatinine clearance	62 [17–90]	43 [21–85]	37 [18–75]	0.08
At discontinuation, ml/min	38 [16–82]	28 [15–52]	22 [12–41]	0.02
24 hrs after discontinuation, ml/min				
C-reactive protein (CRP)	135 [35–267]	89 [22–260]	80 [16–172]	0.09
At discontinuation, mg/L	125 [26–238]	100 [20–306]	65 [13–152]	0.28
24 hrs after discontinuation, mg/L				

The baseline characteristics of the three groups, REC, NREC-H, and NREC-C, are summarized in Table 1. The median age, sex, and number of comorbidities were similar among the groups. Table 2 shows the variables at the time of and following the discontinuation of CRRT. The REC group had significantly lower uNGAL levels at 0, 6, and 12 hours after CRRT discontinuation compared to the NREC-A group. The average UO6, UO12, UOC24post, and UOC24pre levels were significantly higher in the REC group than in the NREC groups. There was a tendency towards lower s-Cr and creatinine clearance at 24 hours after CRRT discontinuation in the REC group compared to the NREC-A group.

A notable difference between the NREC-H and NREC-C groups is that the NREC-H group displayed an 8-fold lower mean uNGAL at 24 hours after CRRT discontinuation. Furthermore, the UOC24pre of the NREC-H group had a 5-fold lower mean value than that of NREC-C group.

Out of the 345 potential samples, 214 (62%) urine samples were obtained for NGAL analysis. Fifty-seven samples were lost because the patient died while on dialysis, 30 samples were not obtained due to anuria, 31 samples were not collected (forgotten), and 13 samples were missed because dialysis was reinitiated less than 24 hours after discontinuation.

The overall ICU mortality was 46% (29 patients). At 3 months after ICU discharge, the mortality rate increased to 55% (38 patients). Among the 31 patients who were alive 3 months after ICU discharge, only

2 required dialysis.

Multivariable logistic regression analysis showed that the only predictive factor for the discontinuation of CRRT was UOA12.

The area under the ROC curve (AUC), NPV, and PPV for the different urine outputs and uNGAL values for the prediction of the successful discontinuation of CRRT are presented in Table 3. Estimates for uNGAL at 12 hours and at 24 hours lacked owing to the missing urine samples (discussed in the previous section). Missing values for uNGAL at 12 hours and at 24 hours could not be interpolated using linear regression since the correlation between uNGAL 6 and 12 hours and uNGAL 12 and 24 hours was poor.

Table 3
Predictive values for neutrophil gelatinase-associated lipocalin and urine output.

	Urine output 24 hrs prior to discontinuation	**Urine output 6 hrs**	**Urine output 12 hrs**	**Urine output 24 hrs after discontinuation**	**NGAL 0 hr**	**NGAL 6 hrs**
AUC	86%	89%	91%	88%	74%	81%
NPV	74%	78%	77%	83%	47%	81%
PPV	84%	71%	92%	90%	65%	80%
Cut-off, Youden index	210 ml	105 ml	288 ml	1260 ml	2371 µg/L	1650 µg/L

Result for urine NGAL at 6 hours and urine output 24 hours prior to discontinuation

The combination of uNGAL at 6 hours and UOC24pre yielded the best NPV. The cut-off for UOC24pre was 210 mL (sensitivity 0.81, specificity 0.86). The cut-off for uNGAL at 6 hours was 1,650 µg/L (sensitivity 0.86, specificity 0.73). The combination of uNGAL at 6 hours and UOC24pre with regard to these cut-off values is presented in Table 4.

Table 4
Predictive values for urine neutrophil gelatinase-associated lipocalin at 6 hours combined with urine output.

Test	**NGAL > 1650 µg/L** ***or*** **urine output < 210 ml**		**NGAL > 1650 µg/L** ***and*** **urine output < 210 ml**	
	Non-recovery	Recovery	Non-recovery	Recovery
Test positive	28	9	23	2
Test negative	1	13	6	20
				202
Predictive values	PPV = 0.76		PPV = 0.92	
	NPV = 0.93		NPV = 0.77	
A positive test outcome indicates that the patient is further dialysis dependent.				
Note that the NPV of the expression, NGAL > 1650 ug/L or urine output < 210 ml, is equivalent to the PPV of the expression, NGAL < 1650 ug/L and urine output > 210 ml. In the latter case, a positive test outcome indicates that the patient is dialysis independent.				

PPV = positive predictive value; NPV = negative predictive value

There is a good correlation between uNGAL at 6 hours and uNGAL at 0 hours (r^2 = 0.81) and between uNGAL at 6 hours and uNGAL at 12 hours (r^2 = 0.79), justifying using interpolation based on the linear regression for obtaining the missing uNGAL 6-hour values. From interpolation based on the uNGAL 0-hour values, one “new” uNGAL 6-hour value was obtained. Likewise, based on the uNGAL 12-hour values, 13 “new” uNGAL 6-hour values were obtained. Thus, the uNGAL 6-hour values were available for the analysis. The 3 missing uNGAL 6-hour values were associated with patients in the NREC-A group, all of whom had a UOC24pre < 100 mL.

Discussion

In this prospective study, it was shown that for ICU patients with complex causes of AKI, urine output in combination with uNGAL was a better predictor of renal function recovery than either applied as a single parameter. Relative to the cessation of CRRT, the best predictions, 92% (continued dialysis dependency) and 93% (renal recovery), were obtained with uNGAL at 6 hours after cessation combined with the cumulated urine output for 24 hours prior to cessation. The main implication of this is that although uNGAL as a single parameter was not shown to be diagnostically superior to urine output, it did add to the predictive ability of the latter. Additionally, uNGAL enabled the prediction of renal function recovery as

soon as 6 hours after CRRT cessation, as opposed to 12–24 hours for urine output as a single parameter. As such, this study may be taken as a proof of concept that uNGAL is a significant paraclinical parameter with regard to the evaluation of renal recovery and continued dialysis dependency in ICU patients.

We arbitrarily chose a “positive” test outcome to associate with continued renal failure (and dialysis dependency), but the cut-offs for uNGAL 6 hours and UOC24pre (Table 4) can easily be applied as predictors of renal recovery as well.

Being able to predict the state of recovery or non-recovery in ICU dialysis patients as early as 6 hours after CRRT discontinuation has several potential benefits. In addition to the benefit of reducing the time a given patient is without obligatory dialysis, there may also be an economic benefit by avoiding unnecessarily replacement of the dialysis filter.

The procedure in the ICU from which the patients in this study were recruited is that only patients who are ready to be discharged may be transferred from CRRT to haemodialysis. It follows that to initiate haemodialysis, the patient must have infection/inflammatory control and no longer require vasopressor or respiratory support. In this situation, the laboratory parameters indicating inflammation, including uNGAL, are expected to be low. From Table 2 this difference between patients in whom haemodialysis was initiated and patients in whom CRRT was re-initiated is particular evident for UOC24pre, UOC24post and partly for uNGAL at 24 hours. If, in theory, it was possible to identify patients in need of haemodialysis at a point before CRRT cessation, the NPV of the combined uNGAL at 6 hours/OUC24pre test in our setting would be 100%, as 1 out of 14 patients with a negative test became permanently haemodialysis dependent (Table 4).

To date, only a very few studies have examined the ability of uNGAL ability to predict renal function recovery after dialysis in critically ill patients. In the study by Yang et al. [14], 102 patients were enrolled, and 8 biomarkers, including uNGAL, were analysed at 24 hours after the discontinuation of dialysis. At 60 days after discontinuation, renal recovery was defined as a serum creatinine level < 0.5 mg/dl (84.2 μmol/L). The ability of uNGAL to predict renal recovery at this time point was poor, with an AUC of 50% [14]. However, Yang et al.’s [14] main purpose for measuring uNGAL was not, as in this study, the determination of the immediate need to restart dialysis but prognosticate lasting damage to the kidney. Another study [Biological Markers of Recovery for the Kidney (BioMark)] by Srisawat et al. [15] also indicated the ability of uNGAL to predict more lasting kidney damage. Finally, a study from 2019 by Stads et al. [16] included 92 patients and tested 7 variables, including uNGAL 2 days after the discontinuation of dialysis. Renal recovery was defined as freedom from dialysis 7 days after discontinuation. Urine NGAL was not significantly ($p = 0.025$) associated with renal recovery. However, it should be considered whether this study has clinical relevance. It is likely that, at 2 days after discontinuation, the physician already had a very good inclination of the need for dialysis.

There are several limitations of our study. The major limitation was the small number of included patients. This has 2 implications. With a limited number of patients in the different subgroups of recovery and non-recovery, the cut-off limits for uNGAL at 6 hours and UOC24pre become less certain, since the

range of values of either inevitably will contain gaps rather than representing an unbroken continuum. With regard to the cut-off values representing the best NPV of the combined parameters, uNGAL at 1,650 μg/L and UOC24pre at 210 mL were flanked by values of 1,327 μg/L and 1,863 μg/L and by 195 and 228 mL, respectively. The true cut-off values for the two parameters may therefore in reality lie close to one of their flanking values at either end. The other uncertainty arising from the limited number of patients is the confidence in the calculated NPV and PPV values given in Table 4. For example, the NPV at 93% is based on the ratio of 13/14 patients and will thus – based on the binomial distribution – have a 90% one-sided lower confidence limit at 75%. In conclusion, a larger study with more included patients is needed to confirm the findings of this study.

Second, a number of urine samples were missed for a multitude of reasons. The impact of this was mitigated by extrapolating 14 out of 51 values for uNGAL at 6 hours from uNGAL at either 0 hours or 12 hours using linear regression. Despite solid statistically significant correlations between uNGAL values at 6 hours with those at 0 hours and at 12 hours, this is clearly a source of uncertainty with regard to the values of uNGAL at 6 hours that were interpolated. Finally, concerning urine sampling, when the patient's urine flow is low, there is a risk of NGAL concentration in the very small volume of urine in the catheter, which could lead to falsely elevated uNGAL values. This is probably the main reason uNGAL at 0 hours (the time of CRRT discontinuation) turned out to be a worse predictor of renal recovery than uNGAL at 6 hours. Perhaps this outcome would have been different if we had used diuretics while the patients were on CRRT.

The main strength of the study is that it focused on the main clinical issue, i.e., the turning point at which the physician acts and discontinues CRRT. How soon can the physician obtain a reliable estimate of whether the patient has regained renal function or whether dialysis has to be reinitiated? The study showed that the use of two variables, uNGAL and urine output, had greater clinical usefulness than the use of either variable as a single test. The generalizability of the study results appears to be quite good, especially because the included AKI patients were selected very broadly. However, the results should be used with care in ICUs in which furosemide is given routinely while the patients are on dialysis.

Conclusions

In the setting of a multidisciplinary ICU with patients who have complex causes underlying acute kidney failure, predictive values of 92 and 93% for either continued dialysis dependency or renal function recovery were obtained with a combination of uNGAL at 6 hours and cumulated urine output for 24 hours prior to discontinuation, with cut-offs of 1,650 μg/L and 210 mL, respectively.

Abbreviations

Renal recovery (REC); Non-recovery, all (NREC-A); Non-recovery, haemodialysis (NREC-H), Non-recovery; CRRT (NREC-C); Cumulated urine output for 24 hours prior to CRRT cessation (UOC24pre); Cumulated urine output for 24 hours after CRRT discontinuation (UOC24post), Average hourly urine output the first 6

hours after discontinuation (UOA6), Average hourly urine output the first 12 hours after cessation (UOA12)

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Danish National Committee on Health Research Ethics (reference number: S-20160015), and the process of collecting and storing patient data was approved by the Danish Data Protection Agency (reference number16/1422). Patient informed consent was obtained according to Danish law.

CONSENT FOR PUBLICATION

We give consent for publication.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

JT collected the data. US and JT contributed to the design, analysis and interpretation of the data and the drafting and revision of the manuscript and gave approval for the final version. PT contributed to the design and approval of the final version.

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Figures

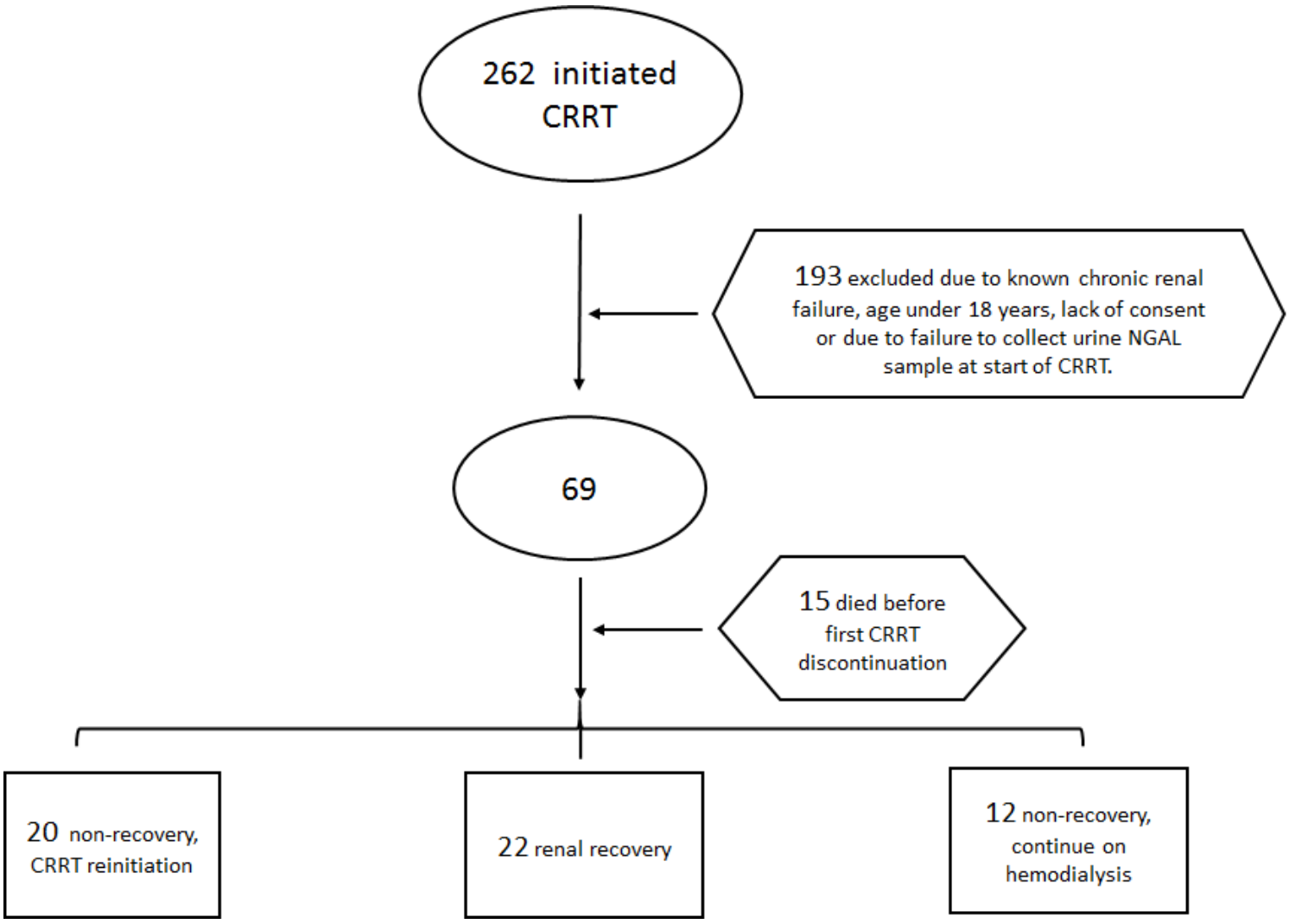

Figure 1

Flow chart of the inclusion of study subjects -no need for legend.