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# Determinants of urinary output response to intravenous furosemide in acute kidney injury: a pharmacokinetic / pharmacodynamic study

**Running title:** Furosemide PK/PD in acute kidney injury

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## ***Abstract***

**Objectives:** This study assessed the determinants of urinary output response to furosemide in acute kidney injury; specifically, whether the response is related to altered pharmacokinetics or pharmacodynamics.

**Design:** Prospective cohort.

**Setting:** Tertiary intensive care unit.

**Patients:** Thirty critically ill patients with acute kidney injury without preexisting renal impairment or recent diuretic exposure.

**Intervention:** A single dose of IV furosemide.

**Measurements and Main Results:** Baseline markers of intravascular volume status were obtained prior to administering furosemide. Six-hour creatinine clearance, hourly plasma/urinary furosemide concentrations, and hourly urinary output were used to assess furosemide pharmacokinetic/pharmacodynamic parameters. Of 30 patients enrolled, 11 had stage-1 (37%), nine had stage-2 (30%) and 10 had stage-3 (33%) Acute Kidney Injury Network acute kidney injury. Seventy-three percent were septic, 47% required norepinephrine, and 53% were mechanically ventilated. Urinary output doubled in 20 patients (67%) following IV furosemide. Measured creatinine clearance was strongly associated with the amount of urinary furosemide excreted and was the only reliable predictor of the urinary output after furosemide (area under the receiver-operating-characteristic curve, 0.75; 95% CI, 0.57-0.93). In addition to an altered pharmacokinetics ( $p < 0.01$ ), a reduced pharmacodynamic response to furosemide also became important when creatinine clearance was reduced to less than  $40 \text{ ml/min/1.73 m}^2$  ( $p = 0.01$ ). Acute kidney injury staging and markers of intravascular volume, including central venous pressure, brain-natriuretic-peptide concentration and fractional urinary sodium excretion were not predictive of urinary output response to furosemide.

**Conclusions:** The severity of acute kidney injury, as reflected by the measured creatinine clearance, alters both pharmacokinetics and pharmacodynamics of furosemide in acute kidney injury, and was the only reliable predictor of the urinary output response to furosemide in acute kidney injury.

**Key words:** *Diuretics, Pharmacology, Prediction, Renal failure, Response*

## Introduction

Acute kidney injury (AKI) is an increasingly common cause and complication of hospital admission and is associated with significant independent morbidity and mortality (1). AKI affects up to 30-50% of critically ill patients, and in the 5% of those requiring renal replacement therapy (RRT), mortality rates are up to 50% (2). Despite this, there remains very little specific therapy that can reduce morbidity and mortality in AKI (3). Evidence suggests that non-oliguric AKI, either spontaneously or in response to diuretics, is associated with a better prognosis than oliguric AKI (4,5). Because oliguria is a risk factor for poor outcomes in AKI and also makes fluid and electrolyte management more difficult, many clinicians use large doses of furosemide – a potent loop diuretic – to increase urine output in AKI (6). Furosemide blocks the activities of the Na-K-Cl<sub>2</sub> co-transporters which may reduce the metabolic demand on the loop of Henle. As such, furosemide has been used by some clinicians in an attempt to reduce the progression of AKI.

The traditional way of managing AKI with intravenous (IV) furosemide may stem from a lack of understanding about the determinants of urinary output response to furosemide, and whether the pharmacokinetics / pharmacodynamics (PK/PD) of furosemide are altered in AKI. Furosemide PK/PD studies in human AKI have not been performed; this may, in part, be due to the fact that standardised definitions of AKI were not available until recently (7,8). Current dosing strategies are therefore commonly based on PK/PD studies performed in patients with severe chronic renal failure, renal transplants, or nephrotic syndrome (9). In patients with severe chronic renal impairment, although the potency of furosemide is reduced (a given dose of furosemide results in a smaller diuresis as reduced renal blood flow means less furosemide is able to be secreted into the lumen of the proximal tubules i.e. a PK limitation) its efficacy in inducing diuresis (reflected by maximal urinary sodium excretion) remains similar to patients with normal renal function (i.e. furosemide PD

remains normal) (9). As such, large doses of furosemide have been recommended to induce diuresis in patients with severe chronic renal impairment (9), and many clinicians have extrapolated this recommendation to patients in AKI.

It is possible that furosemide PK/PD may be different between patients with AKI and chronic renal impairment (10). Administering large doses of furosemide in AKI may be harmful, with possible adverse effects including ototoxicity and increased risk of renal impairment (11,12). In addition, the unsuccessful implementation of furosemide therapy in AKI may delay RRT, and this has been associated with increased mortality (13,14). In this study, we aimed to assess the determinants of the urinary output response to IV furosemide in patients with AKI, including the relative contributions of PK and PD factors and how these may be altered in relation to the severity of AKI.

## **Materials and Methods**

After obtaining ethics approval (EC2011/130) and written informed consent from all patients or from their next-of-kin, 30 patients admitted to the high dependency and intensive care units of Royal Perth Hospital, between March 2013 and October 2014, were prospectively recruited. Patients with AKI according to the Acute Kidney Injury Network (AKIN) criteria (8) were eligible for recruitment if they were judged by their treating intensivist to require a dose of IV furosemide to increase urine output. Only patients without pre-existing chronic kidney disease were eligible. More detailed inclusion and exclusion criteria are detailed in **Supplemental Digital Content S1** (15,16).

### ***Study Protocol***

Demographic data recorded for each patient included height, weight, age, diagnosis, comorbidities, AKIN AKI staging, Acute Physiology and Chronic Health Evaluation

(APACHE) II and Sequential Organ Failure Assessment (SOFA) scores. In addition, peak plasma urea and creatinine concentrations during hospital admission, plasma urea and creatinine concentrations on hospital discharge (or death), requirement for and duration of RRT, and hospital mortality were recorded. Continuous veno-venous hemodiafiltration was the only mode of RRT used for the study patients.

The dose of IV furosemide administered to each patient as a bolus, as well as any intravenous fluid administration during the study period was determined by the treating intensivist. Physiological measurements were performed at baseline (immediately prior to administration of IV furosemide; time zero [T0]) and hourly for six hours following furosemide (T1-6). The data collected at each time point are listed in **Supplemental Digital Content S2**. Although plasma creatinine concentration is frequently used to quantify renal function and the severity of renal impairment, it can be difficult to interpret in patients with unstable renal function (17). Two-, four- and six-hour creatinine clearance (CrCl) have, however, been shown to closely correlate with 24-hour CrCl for diagnosis of AKI (18,19). Because six-hour CrCl is not affected by a single dose of furosemide (20), we chose to measure six-hour CrCl (using T6 plasma creatinine concentration and the urine produced between T1 and T6) as a marker of renal function in this study.

As a sensitivity analysis, the relationship between the measured six-hour CrCl and baseline CrCl (based on T0 urine output, plasma and urinary creatinine concentration in the hour immediately before the administration of furosemide) was assessed, and found to correlate with each other closely ( $r=0.79$ , 95% CI: 0.60-0.90;  $p<0.01$ ). In this study, furosemide concentration was measured by ultra-high performance liquid chromatography-mass spectrometry with a Kinetex XB-C18 column (Phenomenex, Torrance, CA) on a Shimadzu Nexera X2 system coupled to a Shimadzu LCMS-8030 triple quadrupole mass spectrometer (Shimadzu, Melbourne, VIC, Australia).

### *Statistical analysis*

A description of the study sample size calculation is provided in **Supplemental Digital Content S3** (21). In the univariate analyses, patients were considered to be ‘responders’ if the ratio of their urine output in the six hours post- vs. pre-furosemide was greater than two (i.e. cumulative urine output in the six hours following furosemide / cumulative urine output in the six hours preceding furosemide >2) (4). As a sensitivity analysis, we assessed whether the performance of the predictors was similar by defining ‘responders’ using an absolute amount of urine output >100ml/hr for at least two hours during the six-hour period after IV furosemide (22). These end-points were used because they have been shown to predict risk of requiring subsequent RRT in patients with AKI in previous studies (4,22). Baseline characteristics were compared between responders and non-responders using Mann-Whitney U, Chi-square or Fisher’s exact test as appropriate.

Six-hour CrCl and baseline CrCl were calculated, as well as fractional urinary excretion of sodium ( $F_{E}Na$ ) before and after furosemide. Total urinary sodium excretion was calculated by multiplying urine sodium concentration in the cumulative urine collected over six hours with the total volume of urine collected over the same period. Hourly plasma and urinary furosemide concentrations were used as an index of furosemide PK, and urine output per microgram of urinary furosemide excretion per hour was used as a marker of PD.

General linear modelling with repeated measures was used to compare plasma furosemide concentrations, urine furosemide excretion, and urine output over the six hours following furosemide between patients with different severity of AKI. A linear mixed model analysis (with random effects of subject and CrCl) was used to assess the independent effects of furosemide PK/PD on urinary output response to IV furosemide after confirming no deviations from normality by visual inspection of the residual plots. For all multivariate



analyses, patients with different severity of AKI were classified according to the measured CrCl ( $<20\text{ml}/\text{min}/1.73\text{m}^2$ ,  $20\text{-}40\text{ml}/\text{min}/1.73\text{m}^2$ ,  $>40\text{ml}/\text{min}/1.73\text{m}^2$ ) for ease of clinical interpretation. Finally, a restricted analysis was conducted on the group of patients who had received IV 40mg furosemide (n=23) to assess whether the results were affected by different doses (despite adjustment by the plasma furosemide concentrations).

## Results

### *Baseline characteristics (Supplemental Digital Content S4)*

Thirty patients (median age 58 years, interquartile range [IQR] 46-75) with a median APACHE II score of 23 (IQR 18-29) and a median SOFA score of 6 (IQR 5-10) were recruited. Sepsis was present in 73% of patients, and 33% had undergone surgery in the seven days prior to recruitment. On the day of testing, 11 patients (37%) had stage 1, nine patients (30%) had stage 2 and ten patients (33%) had stage 3 AKIN AKI. The median time from onset of AKI to enrolment was 43 hours (IQR 24-75).

### *Clinical predictors of urine output response*

Twenty patients (67%) were 'responders', with a median increase in urine output of 200% (IQR 140-650%), compared to ten 'non-responders' who only increased their urine output by 30% (IQR -30 to 70%) in the six hours following a single bolus dose (20-80mg) of IV furosemide (**Supplemental Digital Content S5**). The most important clinical predictor of the urinary output response to IV furosemide was the measured CrCl (area under the receiver-operating-characteristic [ROC] curves for measured six-hour CrCl and baseline CrCl to predict a doubling of urinary output after furosemide were 0.75, 95% CI: 0.57-0.93, and 0.78, 95% CI: 0.59-0.97, respectively). ROC curves for measured six-hour CrCl and serum

creatinine to predict a doubling of urinary output after furosemide are presented in

### **Supplemental Digital Content S6.**

In the sensitivity analysis, using the alternative definition of response to furosemide, 20 patients were considered as ‘responders’. Again, the measured CrCl was the most important predictor (area under the ROC curves for measured six-hour CrCl and baseline CrCl to predict urine output >100ml/hr for at least two hours after furosemide were 0.94, 95% CI: 0.83-1.04, and 0.89, 95% CI: 0.72-1.06, respectively).

There was no significant difference in plasma furosemide concentrations between patients with different levels of CrCl ( $p=0.11$ ), but as CrCl progressively reduced there was a corresponding reduction in amount of urinary furosemide ( $p<0.01$ ) as well as volume of urine excreted ( $p<0.01$ ) (**Figs. 1A-C**). As expected, CrCl was strongly associated with urinary sodium excretion following furosemide ( $p<0.01$ ; **Supplemental Digital Content S7**). AKIN AKI staging at the time of study enrolment and commonly used markers of tissue perfusion or intravascular volume status, including baseline plasma lactate concentrations, fractional urinary sodium excretion, central venous pressure, brain-natriuretic-peptide concentrations, and plethysmographic-variability-index were not predictive of urinary output response to furosemide in AKI (**Supplemental Digital Content S4**). The subsequent renal outcomes of the responders and non-responders to IV furosemide are described in **Supplemental Digital Content S5**.

### ***Altered PK/PD of furosemide in AKI***

The severity of AKI - as described by the measured CrCl - had a linear relationship with the amount of furosemide excreted in the urine ( $r=0.65$ , 95% CI: 0.38-0.82;  $p<0.01$ ; **Fig. 2**). In patients with more severe AKI, renal furosemide clearance was reduced and plasma

half-life was longer. The changes in furosemide PK between patients with different severity of AKI are summarized in **Table 1** (23).

In addition to an altered PK, there was also an altered PD (urinary output) response in patients with severe AKI (**Fig. 3**), as evidenced by a lower hourly urine output per microgram of furosemide excreted into the urine in patients with  $\text{CrCl} < 20 \text{ml/min/1.73m}^2$ . This result was confirmed and quantified by the linear mixed model (**Table 2**). When  $\text{CrCl}$  was  $> 40 \text{ml/min/1.73m}^2$ , the urine output response was primarily determined by the amount of furosemide excreted into the urine (i.e. a PK limitation) ( $p < 0.01$ ). With moderately severe AKI ( $\text{CrCl} 20\text{-}40 \text{ml/min/1.73m}^2$ ), a PD limitation also became important, as evidenced by the significant interaction term between  $\text{CrCl}$  and urinary furosemide excretion ( $p = 0.01$ ). In severe AKI ( $\text{CrCl} < 20 \text{ml/min/1.73m}^2$ ), a PD limitation became an independent factor (in addition to PK changes) in determining urinary output response to IV furosemide ( $p = 0.03$ ). These results remained unchanged when the analysis was restricted to only those who had received 40mg IV furosemide.

## Discussion

This is the first study specifically investigating furosemide PK/PD in patients with AKI. In this prospective study of 30 critically ill patients with AKI, the diuretic effect of a single bolus dose of IV furosemide was best predicted by measured  $\text{CrCl}$ . The relative importance of PK/PD effects in influencing the urinary response to furosemide differed according to the severity of AKI. Commonly used markers of tissue perfusion and intravascular volume status were not predictive of the urinary output response.

Our findings suggested that the severity of AKI has different effects on different parts of the renal tubules. Furosemide is a highly protein-bound ( $> 98\%$ ), weak organic acid which is actively secreted into the urine by organic acid transporters (OATs) in the proximal tubules

(24,25). In patients with elevated plasma urea and creatinine concentrations, uremic acids may theoretically alter furosemide PK by competing for tubular secretion by OATs (26). Recent evidence also suggests that there are substantial structural and functional changes in the proximal tubules in AKI (27-29). In addition, there is a loss of epithelial polarity in renal ischemia-reperfusion injury and inflammation, with redistribution of  $\text{Na}^+/\text{K}^+$ -ATPase from the basolateral to apical membrane (28), reducing the sodium gradient available for secondary active transport of organic acids and thus urinary furosemide excretion. Our results are consistent with these earlier studies and suggest that OATs in the proximal tubules are impaired in proportion to the reduction in  $\text{CrCl}$ , resulting in derangements in furosemide PK in AKI.

AKI has traditionally been defined as acute tubular necrosis, which may in some patients include histological evidence of necrosis in the loop of Henle (30,31). Experimental models of AKI caused by renal ischemia-reperfusion injury or inflammation also show a reduction in the expression of epithelial  $\text{Na}^+/\text{K}^+$ -ATPase and  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  co-transporters (27,29). Our results are consistent with these previous studies; while the proximal tubular function is impaired in mild AKI, loop of Henle function becomes progressively more affected as the severity of AKI increases, resulting in a corresponding PD limitation. Because urinary microscopic analysis was not included as part of our study protocol, we could not confirm whether those with severe AKI in this study had structural tubular damage or just functional impairment in the loop of Henle.

Our findings have multiple implications for clinicians managing patients with oliguric AKI, most importantly when deciding whether or not to administer furosemide and, if so, at what dose. Until now, the only evidence to guide dose selection has been based on studies performed in patients with chronic renal impairment where large doses of furosemide are used to overcome PK limitations. Our results suggest that in patients with less severe AKI

(e.g. CrCl  $>20\text{ml}/\text{min}/1.73\text{m}^2$ ) it is likely to be possible to increase the diuretic response to furosemide if urinary furosemide excretion can be increased, for example by administering larger doses or commencing an infusion. However, due to the strong relationship we demonstrate between AKI severity and diuretic response, it is likely that once CrCl falls to a certain level (e.g. CrCl  $<20\text{ml}/\text{min}/1.73\text{m}^2$ ) administration of further furosemide is unlikely to induce a significant diuresis due to PD limitations. As a delay in commencing RRT increases mortality (13,14), clinicians should be mindful of this PD limitation when attempting to induce diuresis in patients with severe AKI. Early referral to a nephrology service or intensive care unit of patients with AKI who do not respond to a furosemide challenge should be considered (22), particularly for patients with severe AKI being managed in facilities without access to RRT. Intravascular volume status had no effect on furosemide responsiveness in patients with adequate tissue perfusion; fluid challenges administered in this setting with the intention of increasing urine output should be avoided. In addition to potentially delaying RRT, excessive fluid administration is associated with many adverse effects (32) and may increase the hazards associated with transferring patients to dialysis facilities (e.g. acute pulmonary edema).

The PD limitations in severe AKI are of relevance to studies investigating possible protective effects of furosemide. The theoretical mechanism by which furosemide may reduce severity of AKI, through reducing metabolic demand on loop of Henle, would require relatively intact PK/PD. Our results may thus explain why a beneficial effect of furosemide on the progression of AKI has not been shown (33), and why renal outcomes are better in those who do respond to furosemide (indicating intact tubular function and milder AKI) (4,22). Patients with less severe AKI are likely to be the most suitable candidates for enrolment in future randomized controlled trials (RCTs) investigating the ability of furosemide to reduce disease progression. As measured CrCl is a more reliable determinant

of furosemide PK/PD, perhaps it represents a more suitable recruitment criterion than plasma creatinine or AKIN stage for future RCTs on potential benefits of furosemide in early AKI.

This study has some strengths and limitations. This is the first human study examining the furosemide PK/PD in patients with AKI without pre-existing renal impairment or recent diuretic exposure, making the interpretation of furosemide PK/PD reliable. However, septic shock was a cause of AKI in many of our patients, and it is possible that furosemide PK/PD may differ in AKI due to other causes (e.g. hepatorenal syndrome). As we used measured CrCl to reflect the severity of AKI, the PK/PD changes we observed should not be used to guide treatment decisions based on a calculated CrCl or plasma creatinine due to their poor performance under non-steady-state conditions (17). Although this observational study allowed different doses of IV furosemide for the study patients based on clinician preferences, our data clearly suggests that plasma furosemide concentrations were not as important as severity of AKI in determining urinary output response to IV furosemide in AKI (**Figs. 1A-C**). Finally, this pharmacological study was not designed to detect a difference in renal outcomes between those with different furosemide PK/PD, and would not detect the predictive ability of some less important clinical variables (e.g. norepinephrine requirement) (4). Thus, whether using interventions to improve furosemide PK in AKI can improve patient-centered outcomes remains unproven.

## **Conclusions**

CrCl was the most important clinical predictor of the diuretic response to a single bolus dose of IV furosemide in patients with AKI. A decrease in measured CrCl was associated with progressive changes in furosemide PK/PD, the sequence of which suggests that patients with mild AKI are more likely to respond to IV furosemide than those with severe AKI. Using large doses of IV furosemide in severe AKI, when both PK and PD of

furosemide are altered, is unlikely to induce significant diuresis or prevent acute tubular necrosis, and may only delay inevitable dialysis.

**Contributors:** BIS and KMH collected and analysed data, and drafted the initial manuscript.

All authors contributed to study design, data interpretation, and manuscript revision. The corresponding author (KMH) confirms that he had full access to all study data and final responsibility for the decision to submit for publication.

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## Tables

**Table 1. The importance of creatinine clearance in the pharmacokinetics of intravenous furosemide in patients with acute kidney injury**

| Pharmacokinetic Parameter       | CrCl                         |                                |                              | p value |
|---------------------------------|------------------------------|--------------------------------|------------------------------|---------|
|                                 | >40ml/min/1.73m <sup>2</sup> | 20-40ml/min/1.73m <sup>2</sup> | <20ml/min/1.73m <sup>2</sup> |         |
| Plasma furosemide concentration | 5.1 (3.5-6.3)                | 9.8 (5.2-11.7)                 | 7.4 (5.9-12.4)               | 0.07    |
| AUC <sub>0-6</sub> , mg.hr/l    |                              |                                |                              |         |
| Urine furosemide excretion, mg  | 11.7 (8.3-13.9)              | 8.2 (4.3-11.9)                 | 1.1 (0.2-1.9)                | <0.01   |
| CL <sub>total</sub> , l/hr      | 6.7 (5.6-7.7)                | 3.7 (2.7-5.5)                  | 2.9 (2.3-5.0)                | 0.02    |
| CL <sub>urine</sub> , l/hr      | 2.1 (1.6-2.5)                | 0.7 (0.4-1.2)                  | 0.1 (0.0-0.2)                | <0.01   |
| CL <sub>metabolic</sub> , l/hr  | 4.3 (4.0-5.6)                | 2.9 (2.2-4.2)                  | 2.8 (2.3-4.9)                | 0.07    |
| Vd, l/kg                        | 0.12 (0.10-0.16)             | 0.12 (0.11-0.17)               | 0.12 (0.07-0.21)             | 0.90    |
| Vd <sub>ss</sub> , l/kg         | 0.11 (0.09-0.12)             | 0.08 (0.06-0.11)               | 0.06 (0.03-0.10)             | 0.13    |
| Half-life, hrs                  | 1.5 (1.2-2.6)                | 2.3 (1.9-4.4)                  | 2.9 (2.2-4.0)                | 0.01    |

AUC, area under the curve. CL, clearance. CrCl, creatinine clearance. Vd, volume of distribution. ss, steady state.

All values represent median (interquartile range) unless stated otherwise.

Non-compartmental analysis (23) was used to estimate furosemide's PK parameters. The logarithmic trapezoidal method was used to calculate the area under furosemide's plasma concentration-time curve (AUC<sub>0-6</sub>). As plasma furosemide concentration was first measured 1 hour following injection, plasma concentration at time 0 was extrapolated using the function  $C_t = C_0 \times e^{-kt}$  with  $t = 1$  and  $k$  (elimination rate constant) calculated from plasma concentrations at times 1 and 2. Final AUC<sub>0-6</sub> was therefore the sum of 'measured' AUC<sub>1-6</sub> and 'extrapolated' AUC<sub>0-1</sub>. Systemic and renal clearance, and apparent volume of distribution at steady state were estimated from AUC<sub>0-∞</sub> using the methods described by Gibaldi (23). Metabolic clearance of furosemide was calculated as the difference between estimated systemic and renal clearance. Half-life was calculated using the formula  $t_{1/2} = \ln(2)/ke_{l1-6}$ . Comparison between groups performed using Kruskal-Wallis test.

**Table 2: Linear mixed model analysis of the relationship between hourly urine output (dependent variable) following intravenous furosemide and predictors**

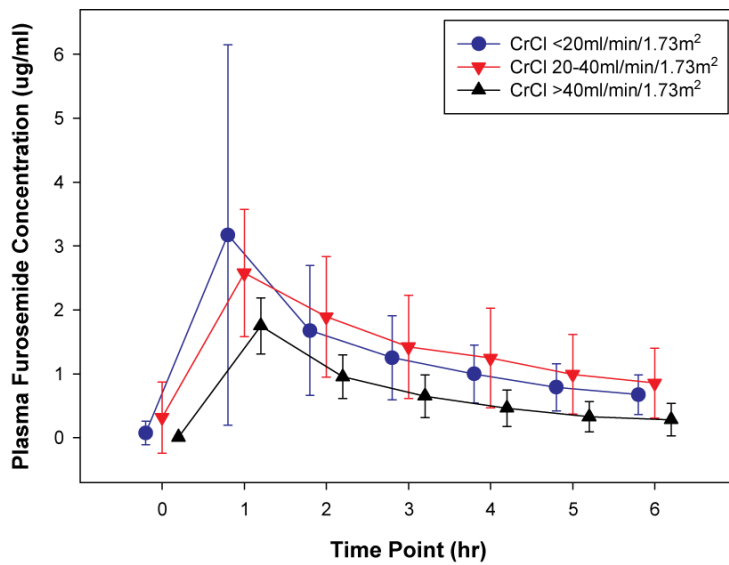
| Predictor  | β coefficient (95% CI) | p value |
|--|------------------------|---------|
| Intercept  | 104 (53, 154)          | <0.01   |
| Time point   | 1.3 (-12, 15)          | 0.85    |
| Hourly urinary furosemide excretion  | 0.07 (0.05, 0.08)      | <0.01   |
| Hourly plasma furosemide concentration                                       | -4.9 (-19, 9.5)        | 0.50    |
| Creatinine clearance   |                        | 0.07    |
| - <20 ml/min/1.73m <sup>2</sup>  | -88 (-167, -9)         | 0.03    |
| - 20-40 ml/min/1.73m <sup>2</sup>  | -14 (-85, 57)          | 0.70    |
| - >40 ml/min/1.73m <sup>2</sup>  | <i>reference group</i> |         |
| Interaction term: creatinine clearance x hourly urinary furosemide excretion |                        |         |
| - <20 ml/min/1.73m <sup>2</sup>  | 0.02 (-0.15, 0.20)     | 0.81    |
| - 20-40 ml/min/1.73m <sup>2</sup>  | 0.03 (0.01, 0.06)      | 0.01    |
| - >40 ml/min/1.73m <sup>2</sup>  | <i>reference group</i> |         |

CI, confidence interval.

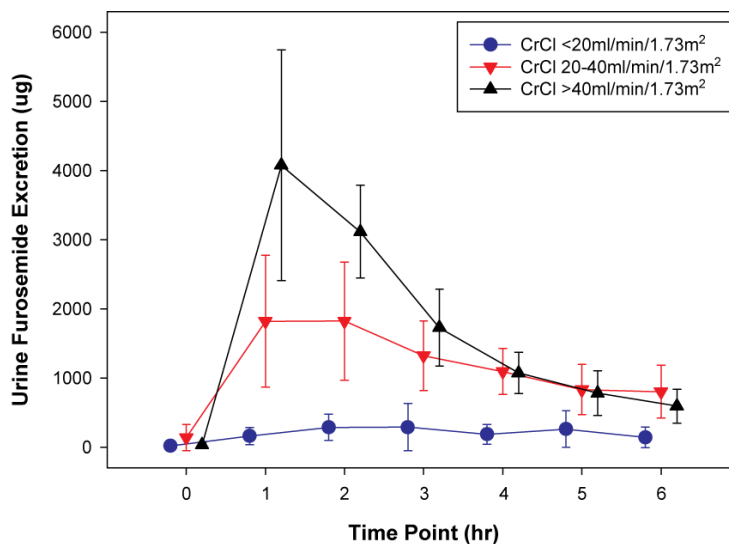
## Figures

**Figure 1:** Changes in hourly (a) plasma furosemide concentration, (b) urinary furosemide excretion and (c) urine output over the six hour period following a single bolus dose of intravenous furosemide, stratified by measured creatinine clearance. Error bars signify 95% confidence interval.

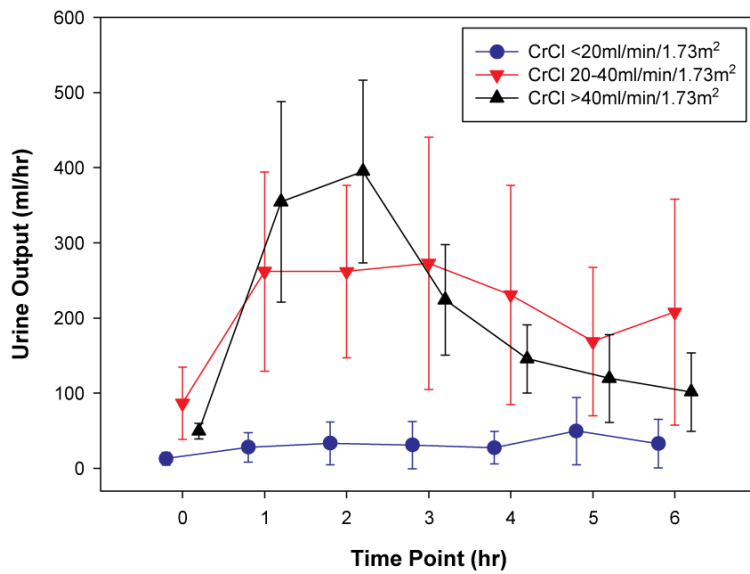
(1A)



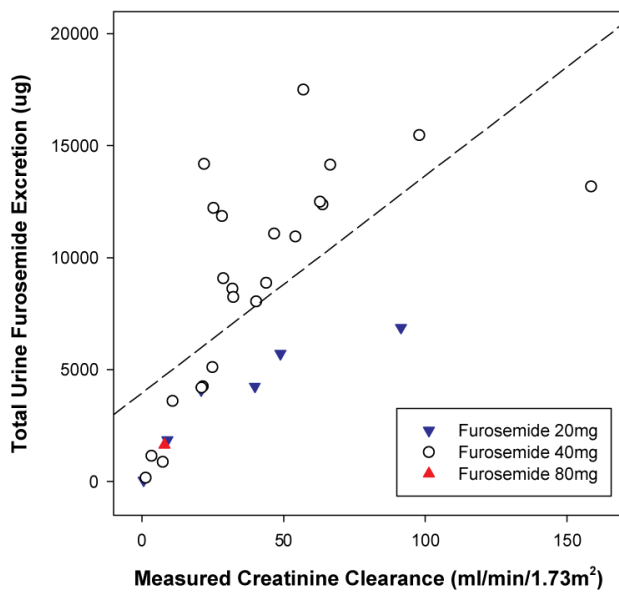
(1B)



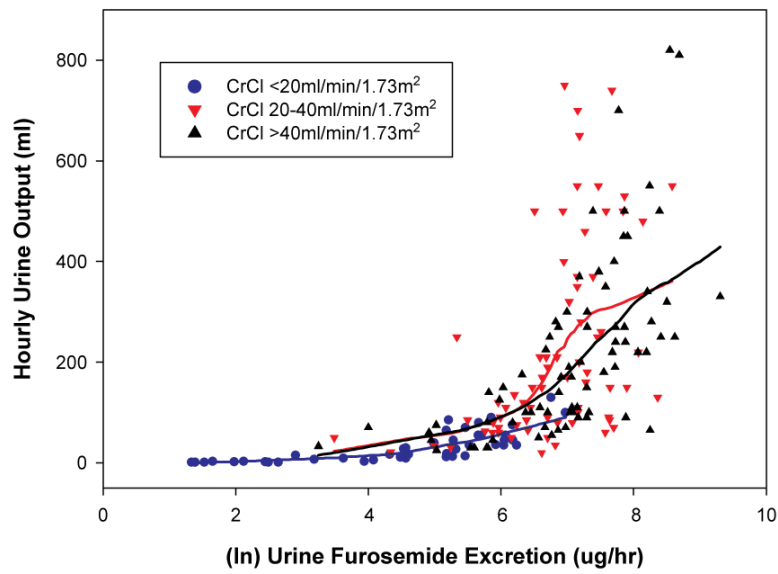
(1C)



**Figure 2:** A relatively linear association was present between measured creatinine clearance and the total amount of furosemide excreted in urine over the six hour period following a single bolus dose of intravenous furosemide. Spearman correlation coefficient 0.80 (95% confidence interval [CI]: 0.62-0.90) and Pearson correlation coefficient 0.65 (95% CI: 0.38-0.82).



**Figure 3:** Relationship between hourly urinary output and hourly urinary furosemide excretion (in natural logarithmic scale), stratified by measured creatinine clearance (CrCl). Trend lines were plotted for each CrCl category using loess regression, and the gradients or slopes of these lines reflect the pharmacodynamic response to furosemide.



## Supplemental Digital Content

### **S1 Inclusion and exclusion criteria for recruitment**

#### Inclusion criteria:

- Patients with acute kidney injury (AKI) (of any stage) according to AKI Network criteria
- Treating intensivist intends to prescribe IV furosemide to increase urine output
- Arterial, central venous and urinary catheters in situ

#### Exclusion criteria:

- Patients with known chronic kidney disease (CKD) (15) or documented estimated glomerular filtration rate (eGFR) <60mL/min (CKD-EPI) (16) in the 3 months prior to current hospital admission
- Treating intensivist intends to prescribe further doses of diuretic medication (including furosemide infusion) within the 6 hours required for study sampling
- Patients who have received intravenous or oral diuretics (including mannitol) in the 24 hours prior to study enrolment
- Patients who have received other medications (e.g. fludrocortisone) known to affect renal sodium or water excretion in the 24 hours prior to study enrolment
- Patients with uncontrolled hyperglycaemia (plasma glucose >10mmol/L)
- Patients receiving renal replacement therapy prior to study enrolment
- Patients with obstructive uropathy, macroscopic haematuria or intra-abdominal hypertension (>20mmHg)
- Age <16 years



## **S2 Description of data collected**

### Hourly data from T0-6:

- Heart rate; mean arterial pressure (transduced from intra-arterial catheter); central venous pressure; noradrenaline infusion dose; mode of ventilation and mean airway pressure; inspired oxygen concentration; hourly urine output; volume and type of fluid boluses administered (if any)
- *Arterial blood sample*: furosemide concentration
- *Urine sample*: furosemide concentration

### Additional data at baseline (T0):

- Plethysmographic variability index (Radical-7, Masimo Corporation, Irvine, California); total urine output in the preceding 6 hours
- *Arterial blood sample*: pH, partial pressures of oxygen and carbon dioxide, bicarbonate, lactate; plasma sodium, potassium, urea, creatinine, albumin, B-type natriuretic peptide, C-reactive protein
- *Urine sample*: sodium, creatinine

### Additional data at T6:

- *Arterial blood sample*: plasma sodium, potassium, urea, creatinine
- *Sample of total urine produced during T0-6*: sodium, creatinine

### Six-hour creatinine clearance (CrCl) measurement:

$$\text{CrCl} = \frac{\text{total urine volume}_{\text{T1-6}} \times \text{creatinine concentration in total urine}_{\text{T1-6}}}{\text{plasma creatinine concentration}_{\text{T6}} \times 360\text{mins}}$$

### Baseline creatinine clearance (CrCl) measurement:

$$\text{CrCl} = \frac{\text{total urine volume}_{\text{T0}} \times \text{creatinine concentration in total urine}_{\text{T0}}}{\text{plasma creatinine concentration}_{\text{T0}} \times 60\text{mins}}$$

### **S3 Sample size calculation**

Following an IV dose of furosemide 40mg, the standard deviation of urinary furosemide excretion (in milligrams) has been reported to range between 1.8 and 9.5 (21). We have previously reported the standard deviation of the ratio of post- vs. pre-furosemide (IV dose range 40-120mg; 80% received 40mg) urine output to be 4.2 in patients with AKI who did not subsequently require dialysis, and 0.46 in patients who did require dialysis (4). Based on this data, the largest sample size that would be required ( $\sigma = 4.2$ ,  $\sigma_x = 1.8$ ) is 32 patients (assuming  $\alpha = 0.05$ ,  $\beta = 0.2$ , and  $\lambda$  [difference in regression line gradient to be detected] = 1.2). However, this is likely to be an over-estimate as the value used for  $\sigma$  will be increased due to the range of furosemide doses used in the original study.

**S4 Differences in baseline characteristics between responders and non-responders to a single bolus dose of intravenous furosemide.**

| Variable   | All patients     | Responders<br>(n=20) | Non-responders<br>(n=10) | p value |
|--|------------------|----------------------|--------------------------|---------|
| Age, years   | 58 (46-75)       | 49 (40-74)           | 65 (53-78)               | 0.08    |
| Body weight, kg  | 89 (73-96)       | 91 (76-98)           | 80 (68-94)               | 0.17    |
| Male, no. (%)  | 19 (63)          | 13 (65)              | 6 (60)                   | 0.90    |
| Sepsis <sup>a</sup> , no. (%)                            | 22 (73)          | 16 (80)              | 6 (60)                   | 0.38    |
| Diabetes mellitus, no. (%)                               | 6 (20)           | 5 (25)               | 1 (10)                   | 0.63    |
| Mechanically ventilated, no. (%)                         | 16 (53)          | 12 (60)              | 4 (40)                   | 0.45    |
| AKIN stage, no. (%):                                     |                  |                      |                          | 0.34    |
| - I  | 11 (37)          | 9 (45)               | 2 (20)                   |         |
| - II   | 9 (30)           | 6 (30)               | 3 (30)                   |         |
| - III  | 10 (33)          | 5 (25)               | 5 (50)                   |         |
| APACHE II score  | 23 (18-29)       | 22 (16-27)           | 27 (18-32)               | 0.23    |
| SOFA score on day of testing                             | 6 (5-10)         | 5 (5-9)              | 9 (5-13)                 | 0.18    |
| <i>Haemodynamic and biochemical:</i>                     |                  |                      |                          |         |
| - Heart rate, beats/min                                  | 80 (72-101)      | 90 (78-107)          | 71 (60-83)               | 0.01    |
| - MAP, mmHg  | 79 (74-96)       | 82 (73-99)           | 78 (74-82)               | 0.45    |
| - CVP, mmHg  | 10 (6-15)        | 11 (7-15)            | 8 (6-15)                 | 0.40    |
| - PVI  | 10 (8-17)        | 10 (9-16)            | 10 (6-21)                | 0.90    |
| - BNP, ng/l  | 322 (72-1425)    | 322 (77-1310)        | 361 (72-1675)            | 0.90    |
| - CRP, mg/l  | 140 (68-250)     | 130 (69-245)         | 140 (63-368)             | 0.75    |
| - Albumin, g/l   | 27 (24-29)       | 27 (25-30)           | 25 (23-27)               | 0.18    |
| - Plasma sodium, mmol/l                                  | 141 (136-148)    | 141 (138-148)        | 142 (132-149)            | 0.75    |
| - Plasma urea, mmol/l                                    | 16 (10-22)       | 14 (9-18)            | 21 (17-26)               | 0.01    |
| - Plasma creatinine, µmol/l                              | 177 (122-262)    | 150 (119-200)        | 241 (159-311)            | 0.07    |
| - Urine output <sup>b</sup> , ml/hr                      | 43 (31-66)       | 48 (35-67)           | 34 (9-63)                | 0.20    |
| - Urine output <sup>c</sup> , ml/kg/hr                   | 0.58 (0.32-0.93) | 0.54 (0.36-0.85)     | 0.61 (0.15-1.26)         | 0.90    |
| - F <sub>ENa</sub> , %                                   | 0.5 (0.2-1.4)    | 0.4 (0.2-1.8)        | 0.6 (0.2-1.1)            | 0.59    |
| - Measured CrCl <sup>d</sup> , ml/min/1.73m <sup>2</sup> | 30 (18-55)       | 40 (22-64)           | 21 (6-33)                | 0.03    |
| - Norepinephrine, µg/min                                 | 0 (0-3.2)        | 0 (0-2)              | 2.8 (0-10.4)             | 0.11    |
| - Plasma lactate, mmol/l                                 | 1.3 (1.0-1.9)    | 1.2 (1.0-1.8)        | 1.4 (1.1-2.5)            | 0.40    |
| IV furosemide dose (20/40/80mg), no.                     | 6/23/1           | 4/15/1               | 2/8/0                    | 0.90    |

AKIN, Acute Kidney Injury Network. APACHE, Acute Physiology and Chronic Health Evaluation. BNP, brain natriuretic peptide. CrCl, creatinine clearance. CRP, C-reactive protein. CVP, central venous pressure. F<sub>ENa</sub>, fractional urinary sodium excretion. MAP, mean arterial pressure. PVI, plethysmographic-variability-index. SOFA, Sequential Organ Failure Assessment.

All values represent median (interquartile range) unless stated otherwise.

<sup>a</sup> Sepsis was defined as documented infection requiring systemic antibiotic treatment at the time of study enrolment.

<sup>b</sup> Median urine output in the hour immediately prior to IV furosemide.

<sup>c</sup> Median hourly urine output in the 6 hours prior to IV furosemide. Total urine output in the 6 hours prior to IV furosemide was also similar between responders and non-responders (p = 0.88).

<sup>d</sup> Creatinine clearance measured over a 6-hour period, area under the receiver-operating-characteristic curve for measured creatinine clearance to predict a doubling of urinary output after furosemide = 0.75 (95% confidence interval [CI] 0.57-0.93). Measured baseline CrCl to predict a doubling of urinary output after furosemide = 0.78 (95% CI: 0.59-0.97).

**S5 Differences in outcomes between responders and non-responders to a single bolus dose of intravenous furosemide**

| Variable   | All patients     | Responders (n=20) | Non-responders (n=10) | p value           |
|--|------------------|-------------------|-----------------------|-------------------|
| Urine output ratio after furosemide <sup>a</sup>                               | 2.4 (1.6-3.6)    | 3.0 (2.4-6.5)     | 1.3 (0.7-1.7)         | <0.01             |
| Urine output after furosemide <sup>b</sup> , l                                 | 0.96 (0.45-1.54) | 1.34 (0.73-1.74)  | 0.45 (0.06-0.85)      | 0.01              |
| Urine output after furosemide <sup>b</sup> , ml/kg/hr                          | 1.96 (0.78-3.34) | 2.60 (1.18-3.38)  | 0.83 (0.11-2.05)      | 0.04              |
| Urinary sodium excretion <sup>b</sup> , mmol                                   | 74 (24-157)      | 132 (38-184)      | 24 (1.2-68)           | 0.01              |
| F <sub>E</sub> Na after furosemide, %  | 3.6 (1.9-6.9)    | 4.0 (2.4-8.1)     | 2.3 (0.7-4.4)         | 0.12              |
| Plasma sodium after furosemide, mmol/l   | 141 (135-147)    | 141 (137-147)     | 143 (133-148)         | 0.81              |
| Total urine output on study day, l   | 2.15 (1.53-3.17) | 2.65 (1.65-3.50)  | 1.71 (0.30-2.13)      | 0.05              |
| Peak plasma urea, mmol/l   | 21 (13-25)       | 17 (12-24)        | 22 (18-28)            | 0.20 <sup>c</sup> |
| Peak plasma creatinine, µmol/l   | 203 (167-343)    | 194 (162-327)     | 309 (170-398)         | 0.37 <sup>c</sup> |
| Hospital discharge urea <sup>d</sup> , mmol/l                                  | 9 (6-13)         | 8 (6-11)          | 11 (5-16)             | 0.59              |
| Hospital discharge creatinine <sup>d</sup> , µmol/l                            | 99 (73-147)      | 99 (77-129)       | 97 (59-184)           | 0.75              |
| Received CRRT, no. (%)   | 6 (20)           | 3 (15)            | 3 (30)                | 0.37              |
| Either received CRRT or had a decision not for dialysis <sup>e</sup> , no. (%) | 8 (27)           | 4 (20)            | 4 (40)                | 0.38              |
| ICU mortality, no. (%)   | 5 (17)           | 2 (10)            | 3 (30)                | 0.30              |
| Hospital mortality, no. (%)  | 6 (20)           | 3 (15)            | 3 (30)                | 0.37              |

CRRT, continuous renal replacement therapy. F<sub>E</sub>Na, fractional urinary sodium excretion. ICU, intensive care unit.

All values represent median (interquartile range) unless stated otherwise.

<sup>a</sup> Calculated as: (total urine output in 6 hours following furosemide) / (total urine output in 6 hours preceding furosemide)

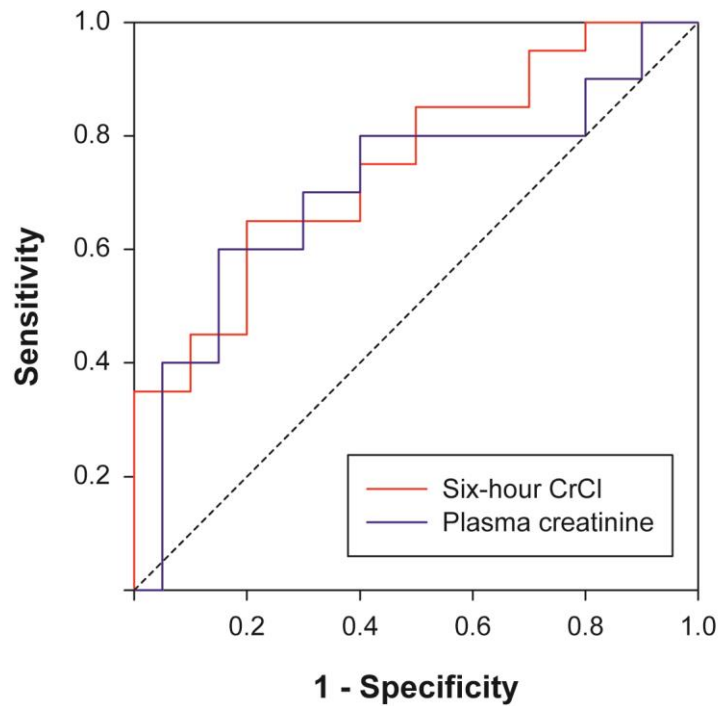
<sup>b</sup> Total in the 6 hours following IV furosemide.

<sup>c</sup> p values for peak plasma urea and creatinine concentrations were 0.06 and 0.76, respectively, when patients who eventually needed dialysis were excluded.

<sup>d</sup> Last recorded value prior to hospital discharge or death.

<sup>e</sup> Includes all patients who received CRRT as well as patients who required CRRT but did not receive it due to treatment limitation orders.

**S6 Receiver operating characteristic (ROC) curves for measured six-hour creatinine clearance (CrCl) and serum creatinine to predict a doubling of urinary output after furosemide**



Area under ROC curve:

Measured six-hour CrCl: 0.75 (95% confidence interval: 0.57-0.93),  $p = 0.03$

Plasma creatinine (T0): 0.71 (95% confidence interval: 0.50-0.93),  $p = 0.07$

**S7 Six-hour urinary sodium excretion over two consecutive periods, before and immediately after a single bolus dose of intravenous furosemide, stratified by measured creatinine clearance. Error bars signify 95% confidence interval.**

