**Urea-to-creatinine ratio trajectories in multi-organ failure: a reanalysis of the REDOXS trial statistical analysis plan**

Ryan W. Haines,1,2 Alex J. Fowler,1,2 Yize I. Wan,1,2 Darren K. Heyland,4 Andrew Day,5 Zudin Puthucheary1,2 & John R. Prowle1,2,3

1Adult Critical Care Unit, The Royal London Hospital, Barts Health NHS Trust, Whitechapel Road, London, E1 1BB, UK

2William Harvey Research Institute, Queen Mary University of London, London, UK

3Department of Renal Medicine and Transplantation, The Royal London Hospital, Barts Health NHS Trust, Whitechapel Road, London, E1 1BB, UK

4Department of Critical Care Medicine, Queen's University, Kingston, Ontario, Canada.

5Department of Community Health and Epidemiology and CERU, Queen's Unversity, Kingston, Ontario, Canada.

**Introduction**

Despite advances in modern medicine, patients suffering from critical illness continue to have an all-cause mortality of approximately 20%1. Disease severity and organ failure, as opposed to admission diagnoses, continue to determine early mortality. Patients that survive either recover, or develop persistent critical illness: an increasing cohort of resource-intensive patients with high mortality and poor return to home rates2-4.

The urea-to creatinine ratio (UCR) has been recently demonstrated to be a biochemical signature of persistent critical illness and its hallmark catabolic state5. The UCR is a better discriminator than premorbid characteristics5, or postulated biological characteristics such as persistent inflammation6,7, or relative immunosuppression8,9. While creatinine is often seen as a marker of renal function, serum creatinine is increasingly understood to better reflect muscle mass, which declines rapidly in critical illness10,11. Creatinine is a byproduct of creatine metabolism, and intracellular creatine content also declines during critical illness, irrespective of nutritional delivery12. Amino acids that are not taken up by skeletal muscle undergo hepatic oxidization, and ammonia converted to urea, which is renally excreted.

Critical illness is characterized by physiological insults that may prevent skeletal muscle uptake of amino acids such as inflammation13, immobilization14, acidosis15 or continuous feeding16, resulting in decreased muscle protein synthesis11,12 (and therefore decreases in muscle mass and serum creatine). In these settings, exogenous amino acids are likely to be oxidized, increasing serum urea. Thus, both inability to utilize ingested protein and muscle breakdown may result in increased urea production during critical illness.

This analysis aims to better understand the determinants of urea and creatinine generation during critical illness and their relationship to outcome. The REDOX Trial randomised 1223 critically ill adults in multi-organ failure in 40 intensive care units (ICUs) worldwide to glutamine supplementation, or placebo within 24h of ICU admission in a 2x2 factorial design with selenium administration. In this study in-hospital and 6 month mortality was greater in those receiving glutamine supplementation17. We hypothesized that an identifiable subgroup of patients exists that did not benefit from exogenous amino acid delivery and that this group would be characterized by increased UCR reflecting increased urea, but decreased creatinine-production. We additionally hypothesized that a dose-dependent relationship would exist between amino acid delivery, urea generation, and thus risk of death.

**Study objectives**

*Hypothesis*

Amongst a heterogenous group of critically ill patients with multiorgan failure, different trajectories of urea:creatinine ratio are associated with different short and long-term outcomes.

*Primary objective*

* To describe 90-day risk of death associated with different urea:creatinine trajectories amongst patients with multi-organ failure.

*Secondary objectives*

* To describe other relevant short and long-term outcomes associated with different urea:creatinine ratio trajectories.
* To describe biochemical, clinical and treatment characteristics associated with different urea:creatinine ratio trajectories.
* To model the determinants of urea:creatinine trajectory during an ICU admission.
* To model the association of different urea:creatinine trajectories with 30 and 90 day mortality.

*Primary outcome*

* 90-day mortality

*Secondary outcomes*

* 30 day, 3 and 6-month mortality
* Length of ICU stay
* 3 and 6-month SF 36 (physical component)
* Days requiring organ support
* Shock free days
* Ventilator free days

**Study population**

This study is a secondary analysis of patients enrolled in a prospective randomized trial to evaluate the efficacy of supplemental glutamine and antioxidant strategies in critically ill patients (REducing Deaths due to OXidative Stress: The REDOXS study, registered at clinicaltrials.gov [NCT00133978](https://clinicaltrials.gov/ct2/show/NCT00133978)). REDOXS included mechanically-ventilated adult patients (≥18 years old) admitted to ICU with two or more of the following organ failures related to their acute illness: 1. A PaO2/FiO2 ratio of ≤300; 2. Clinical evidence of hypoperfusion defined as the need for vasopressor agents (norepinephrine, epinephrine, vasopressin, ≥5 μg/kg/minute of dopamine, or ≥50 μg/minute phenylephrine) for greater than or equal to two hours; 3. In patients without known renal disease, renal dysfunction defined as a serum creatinine ≥171 μmol/L or a urine output of less than 500 ml/last 24 hours (or 80 ml/last 4 hours if a 24-hour period of observation not available). In patients with acute on chronic renal failure (pre-dialysis), an absolute increase of ≥80 μmol/L from baseline or pre-admission creatinine or a urine output of <500 ml/last 24 hours (or 80 ml/last 4 hours) is required; 4. A platelet count of ≤50 × 109/L. Detailed methodology are described elsewhere.17

*Inclusion criteria*

* The primary analysis will only include patients surviving to day 7. Patients who do not survive will have fewer available blood results and may therefore impact the urea:creatinine trajectory analysis.
* The REDOXS trial results report a large impact of glutamine on ureagenesis. For this reason, we will consider glutamine and non-glutamine groups separately.

*Definitions*

Days requiring organ support is reported as need for; mechanical ventilation, renal replacement therapy (RRT) and vasopressors. A sum of each organ support for each day of ICU admission will be calculated. The resulting score compared between clusters. The REDOXS database contains information on organ support up to and including day 28. For example, a patient who is admitted to ICU for 10 days and requires mechanical ventilation and vasopressors for 8 out of 10 days will score 16. If the patient dies before day 10, each day up to day 28 will be scored as maximal organ failure (3 points per day).

*Variables*

Definitions used in REDOXS will be used for this analysis. Additional variables will be derived from REDOXS dataset. The following variables will be considered between urea:creatinine ratio trajectories:

* Age
* Sex
* Charlson comorbidity score
* Admission severity of illness scores (APACHE, SOFA)
* Admission primary diagnosis
* Trial arm
* ICU acquired infection
* Antibiotic use (days alive receiving antibiotics)
* Temperature
* White blood cell count
* Protein delivered
* Calories delivered
* PF ratio

**Statistical considerations**

*Sample size*

No sample size calculation has been performed. The number of patients meeting the above inclusion criteria will determine the sample size of this prospectively designed analysis of trial data.

*Association between urea:creatinine ratio and outcomes*

To describe different outcomes in patients with different trajectories of urea:creatinine ratios we will use two approaches:

* Using an unsupervised machine learning technique, we will perform k-means trajectory clustering based on repeated urea:creatinine ratios18. Once we have assigned patients to each cluster we will compare primary and secondary outcomes between clusters. For the primary outcome, Kaplan Meier plots will be created for each cluster up to 3 months. Clustering will be performed separately on both glutamine and non-glutamine patients. Within clusters, we will then describe the association of biochemical and clinical characteristics.
* In a prespecified approach, to examine the determinants of urea:creatinine ratio we will build a linear mixed effects model to test difference in urea:creatinine trajectory. Mixed effects models with random intercept and slope allows for random variation in urea:creatinine ratio at the start of ICU admission and in the rate of change over time. In addition, correlated residuals allow for correlation between one patients measurement at different time points. The linear mixed model will give us a table of coefficients and a better understanding of the contribution of various factors to the urea:creatinine trajectory, table 2. The relationship between logUCR and time will be explored and, if non-linear, spline functions or alternatives will be considered. This information can then be combined with a survival analysis to assess the link between urea:creatinine ratio measurements during the REDOXS study and mortality.

A directed acyclic graph (DAG) was developed from current evidence and scientific reasoning (Fig. 2) to examine the causal association between outcomes and urea:creatinine ratio. Arrows represent potential causal pathways between variables. The chosen outcome variable is mortality. We used the DAGitty R package and website (http://dagitty.net) to inspect the DAG and decide on which variables to adjust for in the analysis. After selecting the desired exposure and outcome, DAGitty automatically provides the minimum set of predictors to adjust for in order to obtain a direct effect pathway.

**Figure 2.** Directed acyclic graph for urea:creatinine (surrogate for catabolism as the exposure) and 90-day mortality (outcome). Green arrows represent causal path. Red arrows represent a confounding path. Blue ovals are ancestors of outcomes. Red markers are ancestors of both exposures and outcomes. Catabolism and persistent catabolism are unobserved (latent) variables (dark grey oval).



Minimal sufficient adjustment sets for estimating the *direct* effect of ucr on mortality in a survival analysis:

* age, aki, glutamine, illness\_severity\_admission., protein, rrt
* aki, comorbidity, glutamine, illness\_severity\_admission., protein, rrt

Linear mixed model example:

* logUCR ~ time + age + aki + RRT + illness\_severity\_admission + glutamine + protein

*Missing data*

For the trajectory analysis we will assess the number of missing urea creatinine ratio results. We expect that missing values will increase over time. At least 2 urea creatinine values will be required for the trajectory analysis. To reduce the impact of missing values we will only include patients who survived to day 7 in the primary analysis. A supplementary table will be provided summarising the number of daily urea:creatinine values. If clusters change when >3 urea:creatinine values are required, we will consider using linear or spline interpolation in the trajectory analysis depending on the pattern of missingness. The linear mixed effects model was chosen as it is robust in handling missing values using maximum likelihood estimation. 19

**Potential tables**

Table: Patient characteristics for each cluster (Glutamine and no glutamine groups)

|  |  |  |  |
| --- | --- | --- | --- |
|  | Cluster A | Cluster B | Cluster C |
| **Age** |  |  |  |
| Mean (SD) |  |  |  |
| Median (IQR) |  |  |  |
| **Sex** |  |  |  |
| Female |  |  |  |
| Male |  |  |  |
| **Admission diagnosis**  |  |  |  |
| SepsisTrauma |  |  |  |
| Other |  |  |  |
| **Charlson comorbidity index** |  |  |  |
| 1 |  |  |  |
| 2 |  |  |  |
| 3 |  |  |  |
| >3 |  |  |  |
| **CCI (median [IQR])** |  |  |  |
| **Illness severity at admission**  |  |  |  |
| APACHE |  |  |  |
| SOFA  |  |  |  |
| ICU acquired infection |  |  |  |
| Antibiotic use |  |  |  |
| Days with temp. >38.0 |  |  |  |
| WBC |  |  |  |
| Protein delivered |  |  |  |
| Calories delivered |  |  |  |
| AKI RRT |  |  |  |
| PF ratio Vent free days Shock free days Days requiring organ support |  |  |  |
| Mortality ICU  |  |  |  |
| ICU LOS  |  |  |  |
| Functional outcomesSF 36 3 month SF 36 6 month  |  |  |  |

Table 2: Linear mixed effects model for urea:creatinine ratio trajectory

|  |  |  |  |
| --- | --- | --- | --- |
|  | coefficent | 95% CI | p-value |
| Age | 0.01 |  |  |
| AKI | -0.2 |  |  |
| Illness severity | +0.2 |  |  |
| RRT | -0.2 |  |  |
| Co-morbidity | 0.01 |  |  |
| … |  |  |  |
|   |  |  |  |
|   |  |  |

**Potential figures**

Figure 1: Flow chart describing patient selection

Figure 2. Trajectory analysis

Figure 3. Linear mixed model predicted trajectory

DAG website code:

dag {

age [pos="-0.567,-0.047"]

aki [pos="-0.607,-0.049"]

catabolism [latent,pos="-0.611,-0.050"]

comorbidity [pos="-0.767,-0.049"]

glutamine [pos="-0.435,-0.053"]

illness\_severity\_admission. [pos="-0.728,-0.050"]

mortality [outcome,pos="-0.269,-0.050"]

persistent\_catabolism [latent,pos="-0.579,-0.051"]

protein [pos="-0.344,-0.053"]

rrt [pos="-0.458,-0.049"]

ucr [exposure,pos="-0.460,-0.050"]

age -> aki

age -> comorbidity

age -> illness\_severity\_admission.

age -> mortality

aki -> illness\_severity\_admission.

aki -> mortality

aki -> rrt

aki -> ucr

catabolism -> persistent\_catabolism

catabolism -> ucr

comorbidity -> aki

comorbidity -> catabolism

comorbidity -> illness\_severity\_admission.

glutamine -> mortality

glutamine -> ucr

illness\_severity\_admission. -> catabolism

illness\_severity\_admission. -> mortality [pos="-0.595,-0.053"]

illness\_severity\_admission. -> protein

persistent\_catabolism -> ucr

protein -> mortality

protein -> ucr

rrt -> catabolism

rrt -> mortality

rrt -> ucr

ucr -> mortality

}

**References**

1. ICNARC. <https://www.icnarc.org/Our-Audit/Audits/Cmp/Reports/Summary-Statistics>.

2. Digital N. Hospital Admitted Patient Care Activity. <https://digital.nhs.uk/data-and-information/publications/statistical/hospital-admitted-patient-care-activity/2017-18>. Published 2017-18. Accessed 20/11/2019, 2019.

3. Hermans G, Van Aerde N, Meersseman P, et al. Five-year mortality and morbidity impact of prolonged versus brief ICU stay: a propensity score matched cohort study. *Thorax.* 2019;74(11):1037-1045.

4. Viglianti EM, Kruser JM, Iwashyna T. The heterogeneity of prolonged ICU hospitalisations. *Thorax.* 2019;74(11):1015-1017.

5. Haines RW, Zolfaghari P, Wan Y, Pearse RM, Puthucheary Z, Prowle JR. Elevated urea-to-creatinine ratio provides a biochemical signature of muscle catabolism and persistent critical illness after major trauma. *Intensive care medicine.* 2019;45(12):1718-1731.

6. Póvoa P. C-reactive protein: a valuable marker of sepsis. *Intensive Care Med.* 2002;28(3):235-243.

7. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Med.* 2017;43(3):304-377.

8. Boomer JS, To K, Chang KC, et al. Immunosuppression in patients who die of sepsis and multiple organ failure. *JAMA.* 2011;306(23):2594-2605.

9. Stortz JA, Mira JC, Raymond SL, et al. Benchmarking clinical outcomes and the immunocatabolic phenotype of chronic critical illness after sepsis in surgical intensive care unit patients. *J Trauma Acute Care Surg.* 2018;84(2):342-349.

10. Kim SW, Jung HW, Kim CH, Kim KI, Chin HJ, Lee H. A New Equation to Estimate Muscle Mass from Creatinine and Cystatin C. *PloS one.* 2016;11(2):e0148495.

11. Puthucheary ZA, Rawal J, McPhail M, et al. Acute skeletal muscle wasting in critical illness. *JAMA.* 2013;310(15):1591-1600.

12. Zudin A Puthucheary RA, Mark M McPhail, Saeed S, Pasha Y Bear DE, Constantin D, Velloso C, Manning S, Calvert L, Mervyn Singer, Rachel L Batterham, Gomez-Romero M, Holmes E, Steiner M, Atherton PJ, Paul Greenhaff, Lindsay Edwards, Kenneth Smith and Stephen D Harridge, Nicholas Hart, Hugh E Montgomery,. The metabolic phenotype of skeletal muscle is acute critical illness. *Thorax.* 2018.

13. Vesali RF, Cibicek N, Jakobsson T, Klaude M, Wernerman J, Rooyackers O. Protein metabolism in leg muscle following an endotoxin injection in healthy volunteers. *Clin Sci (Lond).* 2009.

14. de Boer MD, Selby A, Atherton P, et al. The temporal responses of protein synthesis, gene expression and cell signalling in human quadriceps muscle and patellar tendon to disuse. *The Journal of physiology.* 2007;585(Pt 1):241-251.

15. Papadoyannakis NJ, Stefanidis CJ, McGeown M. The effect of the correction of metabolic acidosis on nitrogen and potassium balance of patients with chronic renal failure. *Am J Clin Nutr.* 1984;40(3):623-627.

16. Bohé J, Low JFA, Wolfe RR, Rennie MJ. Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *The Journal of physiology.* 2001;532(2):575-579.

17. Heyland D, Muscedere J, Wischmeyer PE, et al. A randomized trial of glutamine and antioxidants in critically ill patients. *N Engl J Med.* 2013;368(16):1489-1497.

18. Genolini C, Falissard B. KmL: a package to cluster longitudinal data. *Comput Methods Programs Biomed.* 2011;104(3):e112-121.

19. Harrell FE. *Regression modeling strategies : with applications to linear models, logistic regression, and survival analysis.* New York ; London: Springer; 2001.