Comparison of Cystatin C and Creatinine in the Assessment of Measured Kidney Function during Critical Illness

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Supplemental Material

Study Methods

Study population and setting

ICU admissions were screened daily by study investigators. Enrolled patients were followed up on alternate days to day 7, day 10, and then at ICU discharge.

Inclusion Criteria:

- Major trauma cohort: Patients ≥18y admitted to ICU and anticipated to be mechanically ventilated for ≥48 hours with a primary admission diagnosis of major trauma.
- Non-trauma cohort: Patients ≥18y admitted to ICU and anticipated to be mechanically ventilated for ≥48 hours without a primary admission diagnosis of major trauma.

Exclusion Criteria:

- Death or discharge from hospital considered highly likely by treating physician within 7 days of ICU admission.
- Any of the following conditions: major traumatic brain injury (Abbreviated Injury Scale head injury score ≥ 5), spinal cord injury with paralysis, lower limb amputation, end stage renal disease or disseminated cancer, lack of independence with activities of daily living or non-ambulatory status prior to admission. (Rationale - exclusion of factors where type of injury or comorbid disease will overwhelming determine functional or renal outcomes.)

Assessment of kidney function

Serum creatinine was measured as part of routine care. Unlike creatinine, cystatin C is produced from all nucleated cells and is less likely to be confounded by acute and chronic effects of ill-health on diet and muscle mass. Analyses were based on absolute levels of creatinine and cystatin C and estimated GFR based on the new CKD-epi formulae.[1] We did not include cystatin C measurements from patients on KRT, only >24h after stopping. Creatinine measured in µmol/L was analysed with isotope dilution mass spectrometry (IDMS) calibrated methods in The Royal London Hospital laboratory using an enzymatic method. Cystatin C was determined with a turbidimetric method (Gentian Cystatin-C UDR-Kit for Beckman-Coulter Synchron and UniCel Systems, Ref A52761, Moss, Norway).

Measured GFR was calculated using iohexol plasma disappearance.[2] 5ml of iohexol was required for clearance studies. Iohexol plasma clearance has been endorsed as a gold standard method for the evaluation of GFR in patients with CKD by the National Institute of health and care excellence (NICE) CKD guideline (G73 1.1.6). Serum iohexol concentration was determined by ultra-high performance liquid chromatography (UHPLC) separation and UV detection using the Acquity® UHPLC system (Waters Corporation, Milford, MA) in Uppsala, Sweden.

For iohexol total coefficient of variation (CV) calculated over a time period of one year was 2% at 25 and 91 mg/L. The total interlaboratory CV in Europe was 4.7% for HPLC based determinations (https://doi.org/10.1515/cclm-2018-1175).[3] For cystatin C, total CV calculated over a time period of one year was 2.5% at 1.11 mg/L. Our samples were measured

over shorter time period (one batch), so CV would be expected to be lower than the yearly CV provided.

The formula for single timepoint iohexol clearance by Jacobsson[4] was used and the distribution volume was calculated as a function of body weight.

Clearance = 1/(t/V + 0.0016) × ln(Q0/V×Ct)

Q0 = total injected amount of iohxeol in mg,
T = time interval between injection and sampling in minutes
Ct = tracer concentration in the sample at the time (T)
V = volume of distribution

The volume of distribution (V) was based on the extracellular volume (V) estimated by the Granerus equation [5] as follows:

- for men V (mL) = 166 x weight (kg) + 2490.
- for women V (mL) = 95 x weight (kg) + 6170.

Muscle Ultrasound
Sequential ultrasound assessment of rectus femoris cross-sectional area (RF_{CSA}) was performed as previously described [6,7]. We used the GE Healthcare Vivid S5 ultrasound device with linear transducer probe. Subjects were examined in the semi-supine position (between 30 and 45 degrees), midline and neutral with the assistance of the bedside nursing staff. The right thigh was the preferred side which was measured and marked 2/3 the distance from the anterior superior iliac spine to the superior patella border. Image acquisition involved localising the rectus femoris through ultrasound imaging perpendicular to the long axis at a previously marked point, ensuring the muscle was not compressed by the ultrasound probe and the entire border visualised. Three consecutive images were taken. Area was determined using the ImageJ image-processing software.

To ensure reproducibility study investigators performed blinded independent measurements on healthy volunteers and correlation coefficient of measurements calculated along with a Bland-Altman plot to assess inter-rater reliability. For measurement reliability, an *a priori* power calculation determined that to compare two observations a minimum of 12 rectus femoris measurements were required, anticipating coefficients between 0.6 and 0.97 and using a significance level of 0.05 and power of 80%. To ensure reproducibility in patients who are critically unwell, 12 measurements were performed on critically ill participants on two occasions separated by at least 15 minutes.

Statistical analysis
In a subgroup of 539 patients with an ICU length of stay of greater than 7 days selected from the dataset of a previous retrospective study[8], mean ICU-discharge CKDEpi creatinine-formula eGFR was 88 ml/min/1.73m2 (SD 39) and CKDEpi cystatin-c formula eGFR was 52 (SD 32), mean difference in eGFR 35 ml/min/1.73m2 (SD of mean difference 24). Conservatively, we aim to confirm a smaller difference of ≥20 ml/min/1.73m2 between creatinine and cystatin-c based eGFR in each patient at hospital discharge. Using a paired analysis, a 90% power and

alpha of 0.025 (to allow examination of primary endpoint in two subpopulations) and inflating for a 20% expected mortality rate, results in a sample size of 30. Accordingly, we would need to recruit 30 trauma and 30 non-trauma patients to examine this endpoint in both sub-groups. We targeted recruitment of 62 patients.

We constructed a linear mixed effects model to assess the impact of time on the difference between eGFRcreat and eGFRcys. Random effects were included to account for correlation within subjects, intercepts (varying baseline values), and slopes (varying trajectories over time). Initially, the impact of time was modelled using a restricted cubic spline with 3 knots to assess for non-linear relationships. After demonstrating a linear relationship from day 1 to around day 30, with dampened increase in eGFR difference thereafter, the cubic spline was removed to allow easy inference about difference in eGFR per day over the first 30 days of ICU stay:

Dependent variable:
Difference between eGFRcreat and eGFRcys

Fixed effects:
Days admitted to ICU

Random effects:
Subjects (intercepts)
Days admitted to ICU (slopes)

A second linear mixed effects model was constructed to evaluate the relationship between rectus femoris cross sectional area and days in hospital:
Dependent variable:
Rectus femoris cross sectional area (log-transformed)

Fixed effects:
Days admitted to ICU

Random effects:
Subjects (intercepts)
Days admitted to ICU (slopes)

R Packages
We used the *nlme*[9] to build models and *ggeffects*[10] package for effects plots. We used the *nephro*[11] for CKD-EPI formulas and *rmcorr*[12] for repeated measures correlation. For the Bayesian analysis we used *brms*[13]. Data handling and plotting was done using the package set *tidyverse*[14].

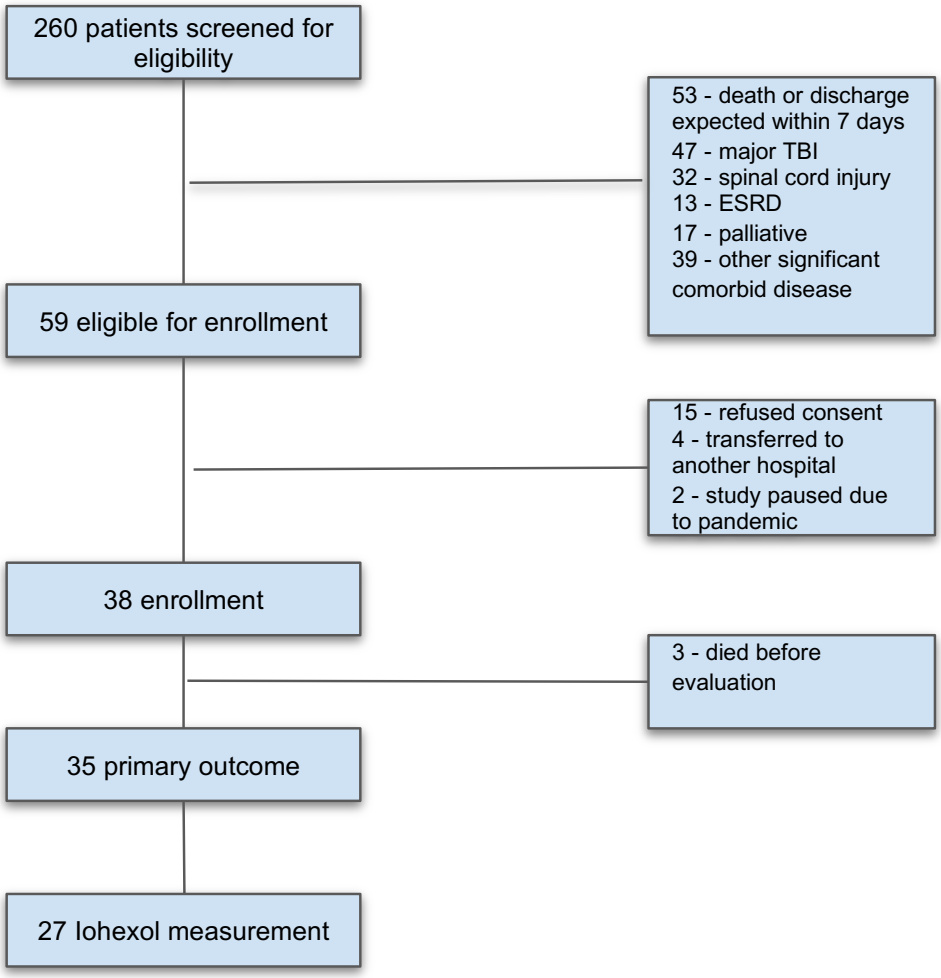
Supplemental Table 1. Difference in eGFR creatinine and eGFR cystatin C at each of study timepoints.

Day	Difference between eGFRcreat and eGFRcys, median [IQR]
1	-4 [-18, 15]
3	0 [-9, 8]
5	13 [-1, 19]
7	13 [0, 26]
10	24 [12, 33]
Day of ICU discharge	30 [14, 52]
Day of ICU discharge + 7days	58 [51, 61]

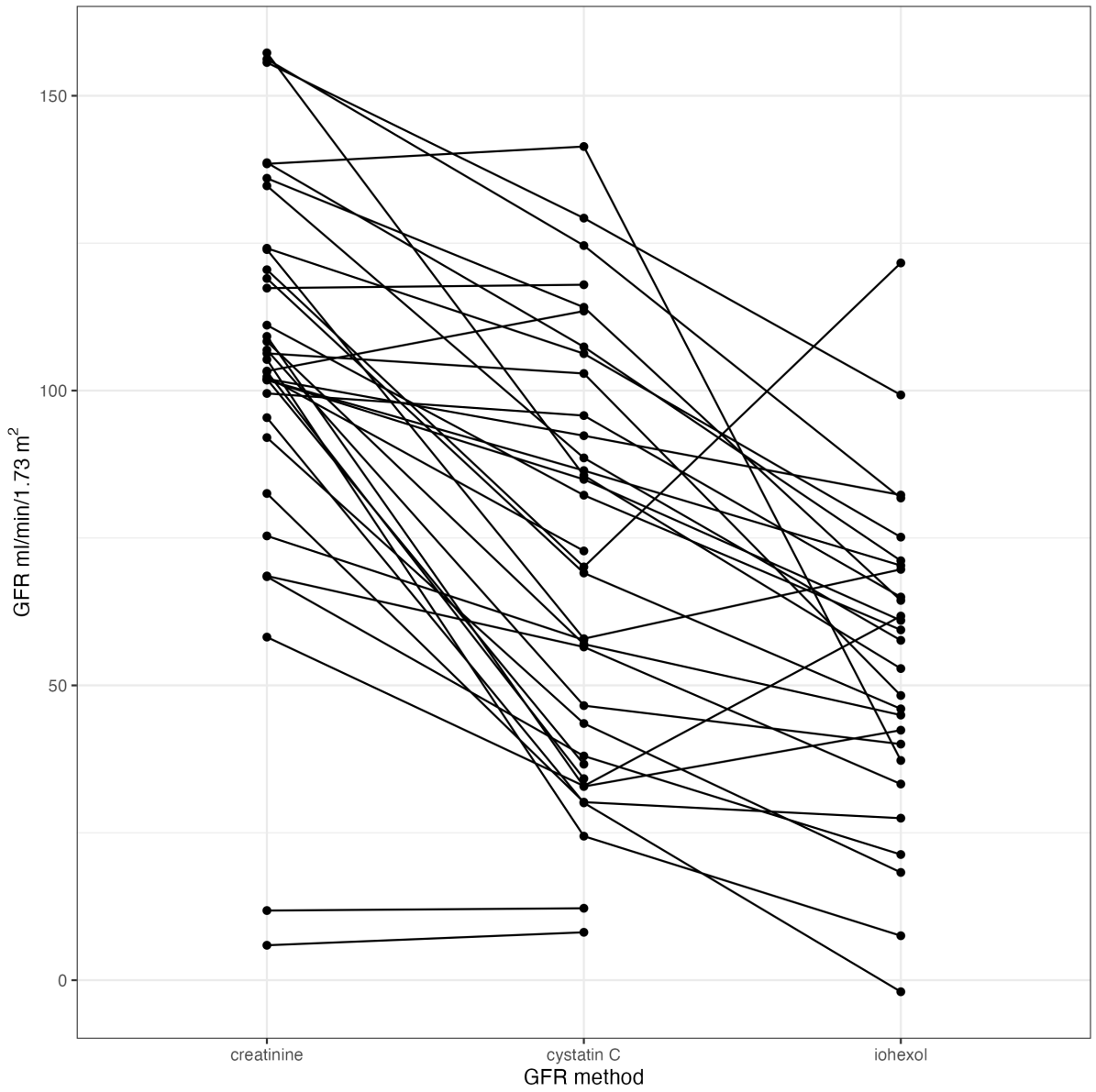
Supplemental Table 2. Linear mixed effects model of change in rectus femoris cross sectional area over time.

Model variable	Beta coefficient (95% CI)
Intercept	1.38 (1.26 – 1.49)
Day of ICU admission (days)	-0.02 (-0.02 – -0.01)

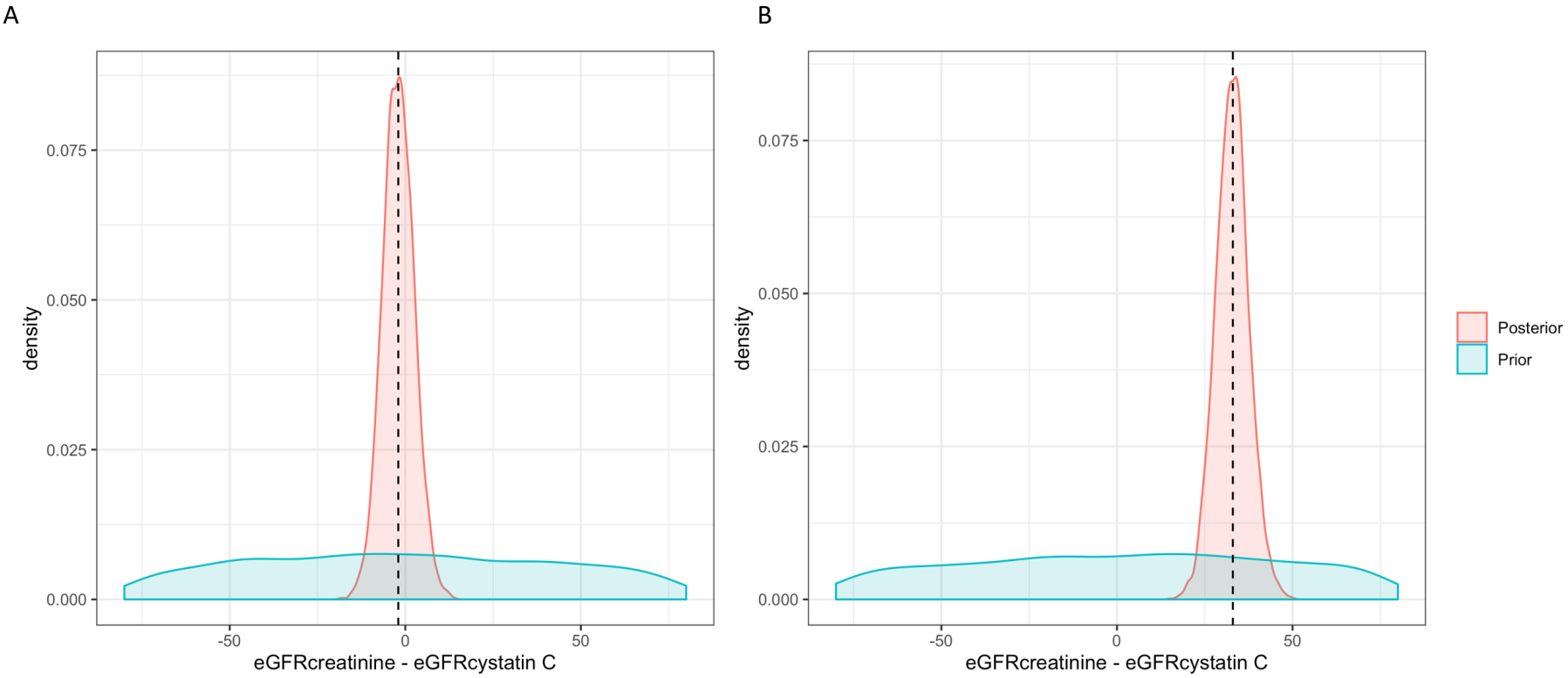
Supplemental Figure 1. Study flow diagram



Supplemental Figure 2. ICU discharge eGFR comparisons, including iohexol measured glomerular filtration rate in (n = 27).

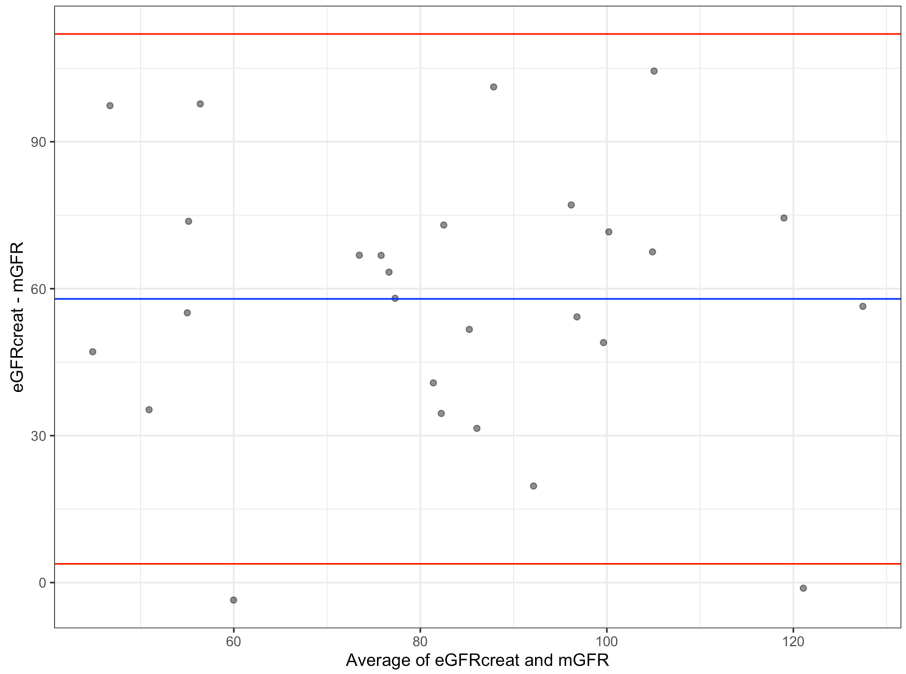


Supplemental Figure 3. Density plot of the prior and posterior distribution of the mean difference in estimated glomerular filtration rate for creatinine and cystatin C at intensive care admission (A) and discharge (B), (n = 35). Dotted vertical line is the point estimate for the mean difference.



Supplemental Figure 4. Bland Altman plots for estimated glomerular filtration rate based on CKD-EPI creatinine (A) and CKD-EPI cystatin C (B) versus measured iohexol glomerular filtration rate. Blue line is mean difference and red lines 95% limits of agreement. *GFR* - glomerular filtration rate (ml/min/1.73m^2)

A



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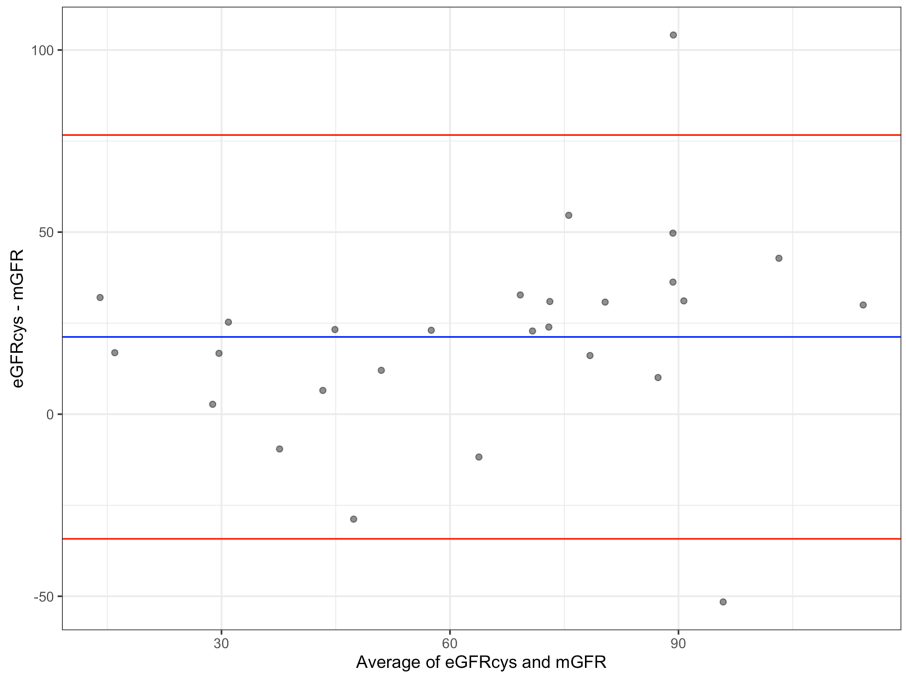
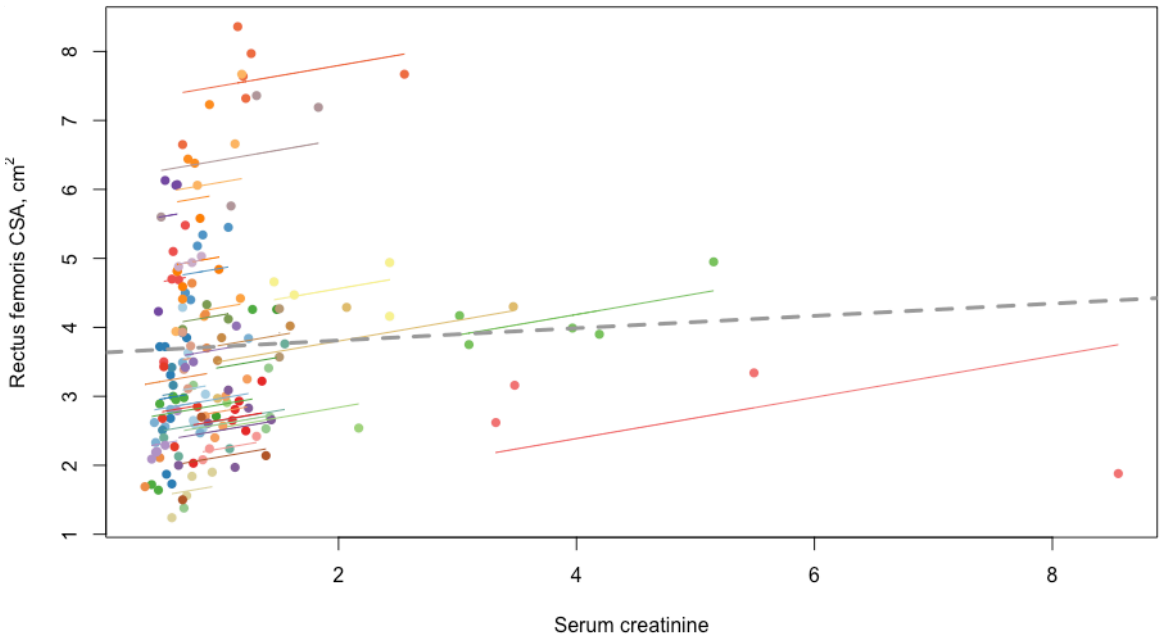
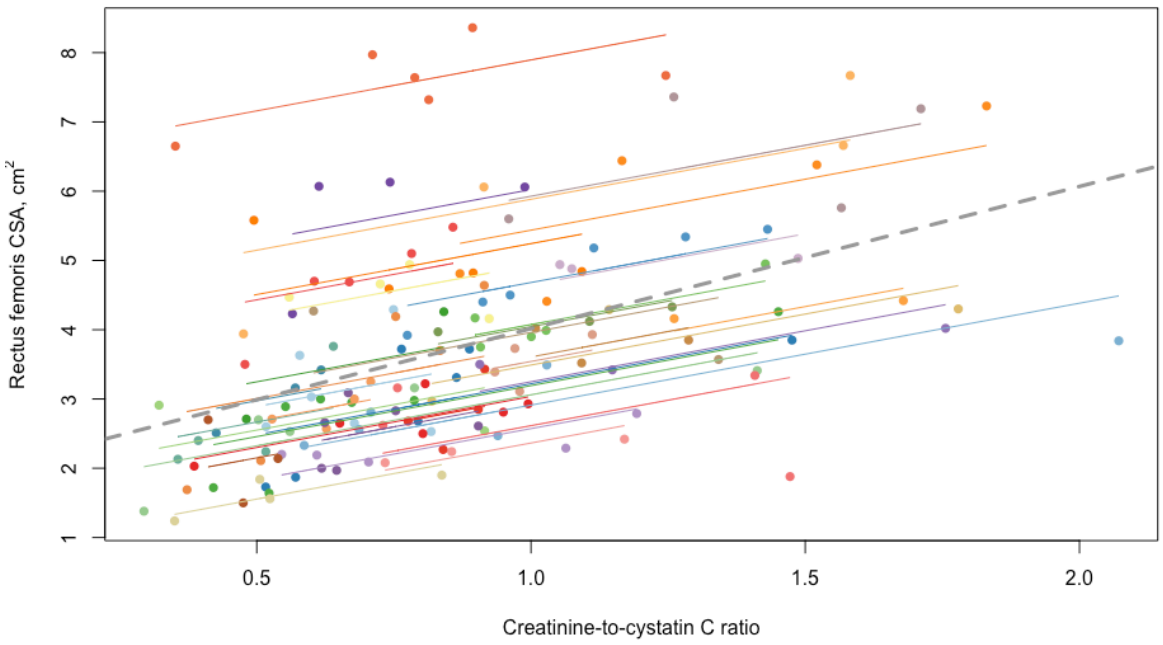


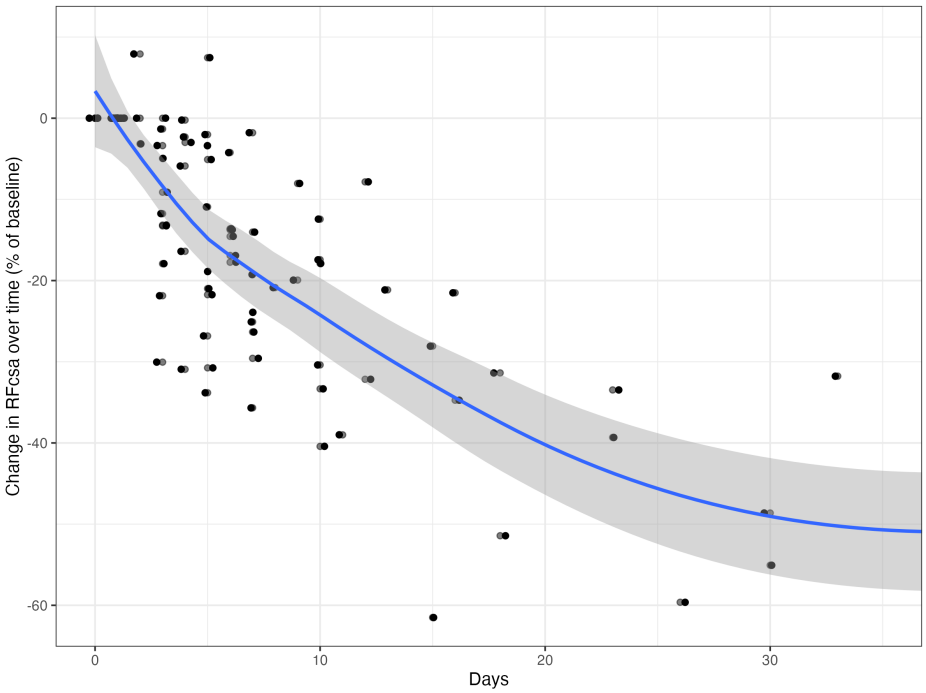
Supplemental Figure 5. Rectus femoris cross sectional area measurements correlated with serum creatinine (A) and creatinine-cystatin C ratio (B). Repeated measures correlation was used for the association calculations. Individuals were coloured differently for comparison.



B



Supplemental Figure 6. Rectus femoris cross sectional area decreased over time in ICU trauma patients. Trend line and confidence intervals using loess smoother.



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