Supplemental content

Methods

PRO-ACT dataset

Our second validation cohort consisted of PRO-ACT data (version Dec. 2015). Within PRO-ACT, plasma creatinine values are given in μmol/L; plasma creatinine values measured originally in mg/dL were transformed by multiplying the values by 88.4. As we showed in the EMPOWER study (**eFigure 1**), these transformations affect the variability due to differences in precision. More importantly, the transformations gave rise to odd longitudinal patterns (i.e. repeating 88.4 μmol/L over an entire 12-month period); it is highly unlikely that these reflect the true biological variation. Therefore, all patients with transformed values from mg/dL to μmol/L were identified and excluded. Additionally, for some patients, a last-observation-carried-forward (LOCF) procedure was performed to account for missing data. As LOCF procedures may bias the estimates, and linear mixed models are flexible in handling missing data, the LOCF procedure was reversed and we removed all imputed values. Finally, we matched each plasma creatinine measurement with an ALSFRS-R score as described in the methods section. Because we found strong evidence that the power of plasma creatinine is severely affected by the sampling interval (i.e. monthly *vs* bimonthly sampling, figure 2), only patients with at least one measurement per 1.15 month (calculated as observed time divided by number of observations) were included in the analysis. A value of 1.15 was chosen to allow for missing data (1.15 corresponds to approximately 2 missing data points for 12 months' follow-up data or 15% missing data). Finally, we merged the matched plasma creatinine and ALSFRS-R scores with baseline characteristics and survival data. If no survival data were available, subjects were censored after their last known follow-up visit.

eFigure 1 title. Differences between measurement scales in the longitudinal variability of plasma creatinine.

eFigure 1 legend. Raw plasma creatinine levels are shown for different follow-up visits during a 12-month period in the EMPOWER study. Plasma creatinine levels, originally determined in milligrams per deciliter, were multiplied by 88.4 to transform the scale to micromoles per liter. As can be seen, original measurements in milligrams per deciliter show considerably less variability between scores and result in a large step size (step size of 8.84 *vs* step size of 1 for μmol/L). Looking at the medians over time (horizontal black lines in the boxplots), this may severely affect the ability of plasma creatinine to detect disease progression.

eTable 1. Variability and longitudinal patterns of the ALSFRS-R and plasma creatinine.

	ALSFRS-R[a]	**Plasma creatinine**[a]
LITRA (n = 50, *μmol/L*)		
Between-patient variability at baseline	0.59 (CI: 0.47 – 0.74)	0.78 (CI: 0.63 – 0.97)
Average slope	-0.11 (CI: -0.13 – -0.09)	-0.07 (CI: -0.09 – -0.06)
Between-patient slope variability	0.06 (CI: 0.04 – 0.08)	0.03 (CI: 0.02 – 0.04)
Within-patient variability	0.27 (CI: 0.24 – 0.31)	0.36 (CI: 0.32 – 0.40)
EMPOWER I (n = 449, *μmol/L*)		
Between-patient variability at baseline	0.63 (CI: 0.59 – 0.67)	0.85 (CI: 0.63 – 0.97)
Average slope	-0.13 (CI: -0.14 – -0.12)	-0.07 (CI: -0.09 – -0.06)
Between-patient slope variability	0.09 (CI: 0.09 – 0.10)	0.05 (CI: 0.02 – 0.04)
Within-patient variability	0.24 (CI: 0.23 – 0.24)	0.39 (CI: 0.38 – 0.40)
EMPOWER II (n = 487, *mg/dL*)		
Between-patient variability at baseline	0.67 (CI: 0.63 – 0.72)	0.82 (CI: 0.69 – 1.21)
Average slope	-0.13 (CI: -0.14 – -0.12)	-0.06 (CI: -0.07 – -0.06)
Between-patient slope variability	0.09 (CI: 0.08 – 0.10)	0.05 (CI: 0.04 – 0.08)
Within-patient variability	0.24 (CI: 0.24 – 0.25)	0.39 (CI: 0.38 – 0.40)
PRO-ACT (n = 255, *μmol/L*)		
Between-patient variability at baseline	0.63 (CI: 0.57 – 0.69)	0.82 (CI: 0.75 – 0.89)
Average slope	-0.14 (CI: -0.16 – -0.13)	-0.07 (CI: -0.07 – -0.06)
Between-patient slope variability	0.11 (CI: 0.10 – 0.13)	0.04 (CI: 0.04 – 0.05)
Within-patient variability	0.24 (CI: 0.23 – 0.25)	0.40 (CI: 0.39 – 0.41)
COMBINED (n = 754, *μmol/L*)		
Between-patient variability at baseline	0.62 (CI: 0.59 – 0.66)	0.83 (CI: 0.79 – 0.88)

Average slope	-0.13 (CI: -0.14 – -0.12)	-0.07 (CI: -0.07 – -0.06)
Between-patient slope variability	0.10 (CI: 0.09 – 0.10)	0.04 (CI: 0.04 – 0.05)
Within-patient variability	0.24 (CI: 0.24 – 0.24)	0.39 (CI: 0.38 – 0.40)

Abbreviations: ALSFRS-R = ALS Functional Rating Scale-revised; CI = 95% confidence interval; Between-patient variability at baseline = random effects standard deviation intercepts; Between-patient slope variability = random effects standard deviation slopes; Within-patient variability = standard deviation residual variance.

[a] Scores were standardized to directly compare the ALSFRS-R total score and plasma creatinine level.

[b] P-values are based on the likelihood ratio test, calculated by merging all outcome data (ALSFRS-R and plasma creatinine data) together and specifying an uncorrelated random effects hierarchy.

eTable 2. Final multivariate Cox PH model after backward stepwise selection.

	Hazard Ratio	95% CI	P-value
Age, y	1.05	1.03 – 1.06	< 0.001
Gender, *ref = female*	2.06	1.51 – 2.82	< 0.001
Site of onset, *ref = bulbar*	0.72	0.54 – 0.97	0.029
ΔFRS, mo[a]	0.15	0.08 – 0.29	< 0.001
Baseline FVC (% predicted)	0.98	0.97 – 0.98	< 0.001
Baseline plasma creatinine (*μmol/L*)	0.98	0.97 – 0.99	< 0.001

95% CI = 95% confidence interval; FVC% = forced vital capacity; [a]ΔFRS = exponent [(ALSFRS-R score at inclusion – 48) / disease duration from symptom onset]; Ref = reference category