

Supplementary materials

Supplementary Table 1. Antibodies used for the ELISA assays for the complement protein measurements

Complement molecule	Capture Ab (clone)	Detection Ab	Secondary Ab	Reference
Ficolin-1	Anti-Fic-1 FCN166	Rabbit anti-Fic-1 HP9039 (Hycult Biotech)	Donkey anti-rabbit-horseradish peroxidase NA09340V (GE Healthcare)	Munthe-Fog L et al. ³⁷
Ficolin-2	Anti-Fic-2 FCN216	Biotinylated anti-Fic-2 FCN219	HRP-conjugated streptavidin (RPN1231V, Sigma)	Munthe-Fog L et al. ³⁸
Ficolin-3	Anti-Fic-3 FCN334	Biotinylated anti-Fic-3 FCN334	HRP-conjugated streptavidin (RPN1231V, Sigma)	Munthe-Fog L et al. ³⁹
Collectin-11	Anti-Collectin-11 HYB17	Biotinylated anti-Collectin-11 HYB15	HRP-conjugated streptavidin (RPN1231V, Sigma)	Bayarri-Olmos R et al. ³⁰
MBL	Anti-MBL HYB131-1	Biotinylated anti-MBL HYB131-1	HRP-conjugated streptavidin (RPN1231V, Sigma)	Garred P et al. ⁴⁰
MASP-3	Anti-MASP-3 7B8	Biotinylated anti-MASP-1/3 7B7	HRP-conjugated streptavidin (RPN1231V, Sigma)	Skjoedt MO et al. ⁴¹
MAP-1	Anti-MAP-1 20C4	Biotinylated anti-MAP-1 8B3	HRP-conjugated streptavidin (RPN1231V, Sigma)	Skjoedt MO et al. ³³
PTX3	Anti-PTX3-20	Biotinylated anti-PTX3-73	HRP-conjugated streptavidin (RPN1231V, Sigma)	Bastrup-Birk S et al. ³¹
C4c	Anti-C4c 99-72-18	Biotinylated anti-human C4c complement	HRP-conjugated streptavidin (RPN1231V, Sigma)	Pilely K et al. ³⁴
C3bc	Anti-C3b/iC3b/C3c bH6	Rabbit anti-human C3c	Donkey anti-rabbit-horseradish peroxidase NA09340V (GE Healthcare)	Garred P et al. ³⁵
sC5b-9	Anti-TCC E11	Rabbit anti-human C9 (104303E)	Donkey anti-rabbit-horseradish peroxidase NA09340V (GE Healthcare)	Mollnes TE et al. ³⁶

Ab: antibody, Anti-Fic-1: ficolin-1 antibody, Anti-Fic-2: ficolin-2 antibody, Anti-fic-3: ficolin-3 antibody, anti-TCC: terminal complement complex antibody, MAP-1: MBL/ficolin/collectin associated protein 1, MASP-3: MBL/ficolin/collectin-associated serine protease 3, MBL: mannose-binding lectin, PTX-3: pentraxin-3

Supplementary Table 2. Results of univariate regression analysis (Pearson's correlation).

Complement component	Sample	Tested on	Output	R²	P
Ficolin-1 (ng/ml)	EDTA plasma	Log Data	ALSFRS-score	0.021	0.17
Ficolin-2 (μg/ml)	EDTA plasma	Log Data	ALSFRS-score	0.011	0.32
Ficolin-3 (μg/ml)	EDTA plasma	Log Data	ALSFRS-score	9.3e-5	0.93
Collectin-11 (ng/ml)	EDTA plasma	Log Data	ALSFRS-score	0.060	0.019
MBL (ng/ml)	EDTA plasma	Sqrt Data	ALSFRS-score	0.0096	0.35
MASP-3 (ng/ml)	EDTA plasma	Log Data	ALSFRS-score	0.011	0.75
MAP-1 (ng/ml)	EDTA plasma	Log Data	ALSFRS-score	0.0020	0.68
C4 (mg/ml)	Serum	Data	ALSFRS-score	0.031	0.095
C4c (AU)	EDTA plasma	Log Data	ALSFRS-score	0.0065	0.45
C3 (mg/ml)	Serum	Log Data	ALSFRS-score	0.073	0.0097
C3bc (AU)	EDTA plasma	Log Data	ALSFRS-score	0.085	0.0051
C3bc/C3	Index	Log Data	ALSFRS-score	0.032	0.090
sC5b-9 (AU)	EDTA plasma	Log Data	ALSFRS-score	0.021	0.17
Ficolin-3 (μg/ml)	CSF	Log Data	ALSFRS-score	0.0077	0.41
C3bc (AU)	CSF	Log Data	ALSFRS-score	0.0052	0.50
C4c (AU)	CSF	Sqrt Data	ALSFRS-score	0.058	0.022

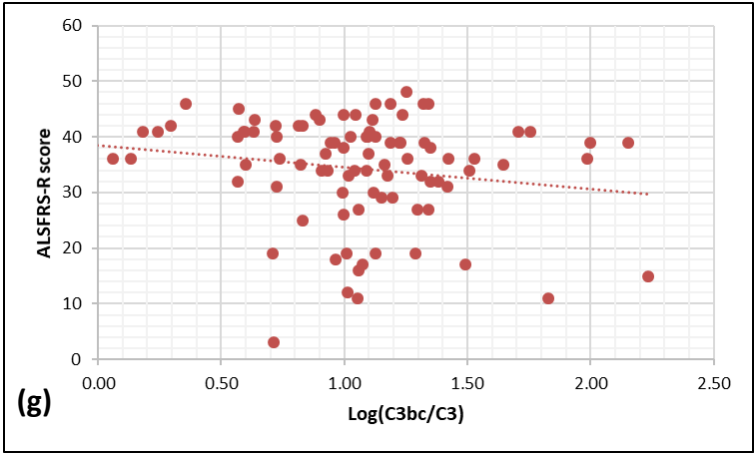
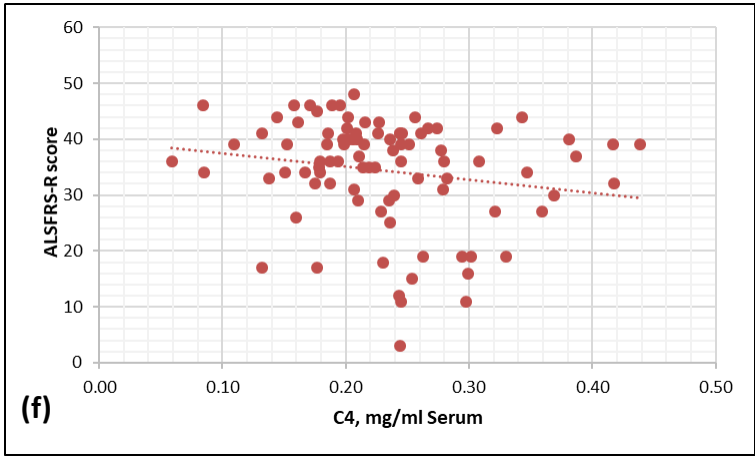
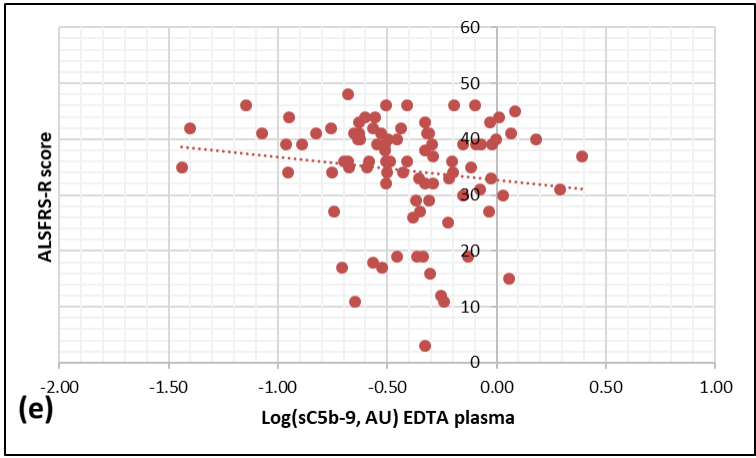
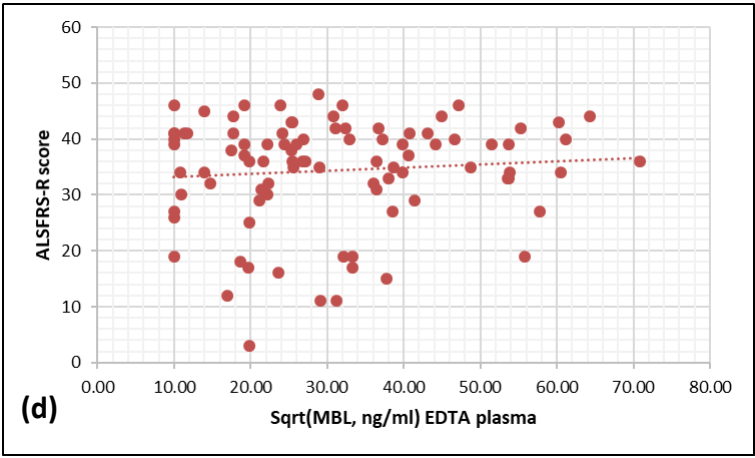
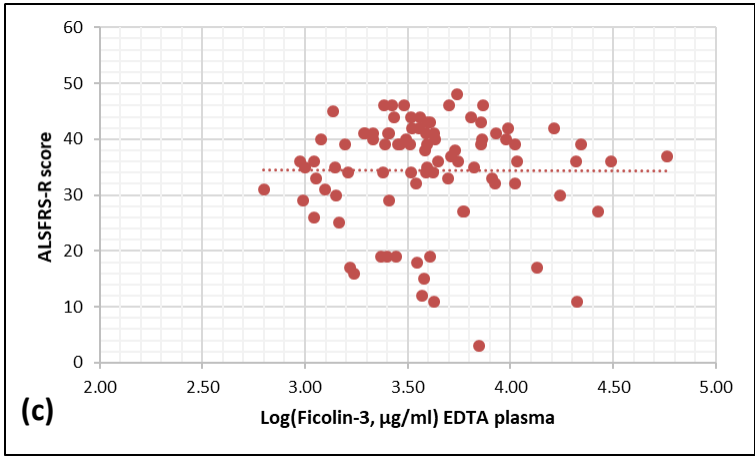
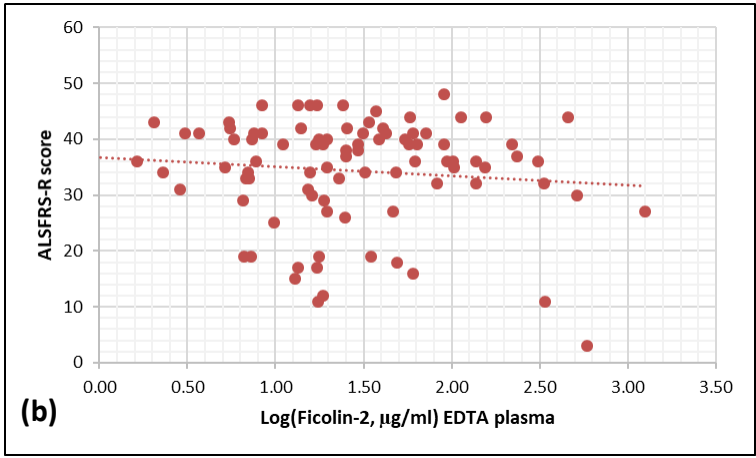
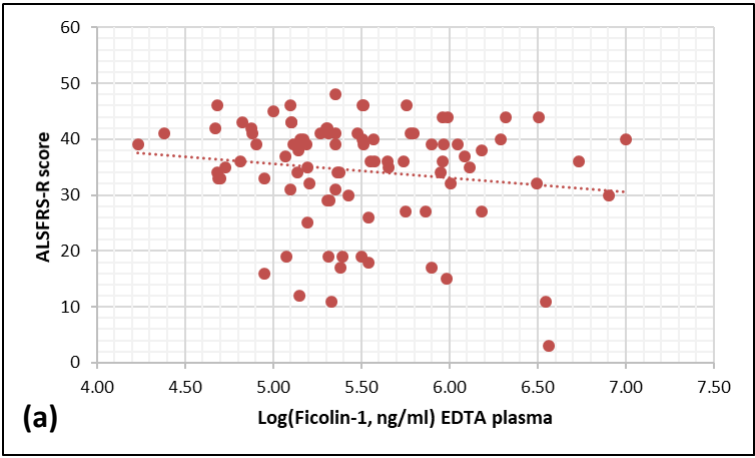
The results of the conducted PTX-3 (EDTA) and sC5b-9 (CSF) assays were not applicable for univariate regression analysis because many results were below the lower limit of detection. AU: arbitrary unit, CSF: cerebrospinal fluid, Log: logarithmically transformed, P: p value, Sqrt: square root transformed.

Supplementary Table 3. Univariate survival analysis for association between median survival and the complement markers ficolin.2 (EDTA), collectin-11 (EDTA), C4 (serum), C3bc (EDTA), C3bc/C3 (fraction), and sC5b-9 estimated by Cox' proportional hazards regression model.

Variable	HR slope	95% CI	P
Ficolin-2 (µg/ml, EDTA)	1.02	0.933-1.11	0.69
Collectin-11 (ng/ml. EDTA)	1.00	0.996 – 1.00	0.97
C4 (mg/ml, serum)	1.89	0.085 – 42.1	0.69
C3bc (AU, EDTA)	1.05	0.887 – 1.25	0.56
C3bc/C3 (fraction)	1.01	0.831 – 1.22	0.96
sC5b-9 (AU, EDTA)	1.32	0.456 – 3.797	0.61
Ficolin-3 (µg/ml, CSF)	1.44×10^{-8}	$3.7 \times 10^{-28} - 5.6 \times 10^{11}$	0.43
C3bc (AU, CSF)	1.003	0.996 – 1.01	0.42

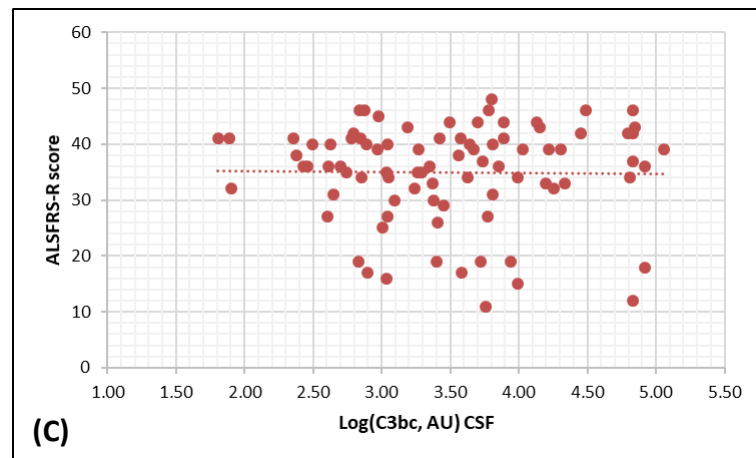
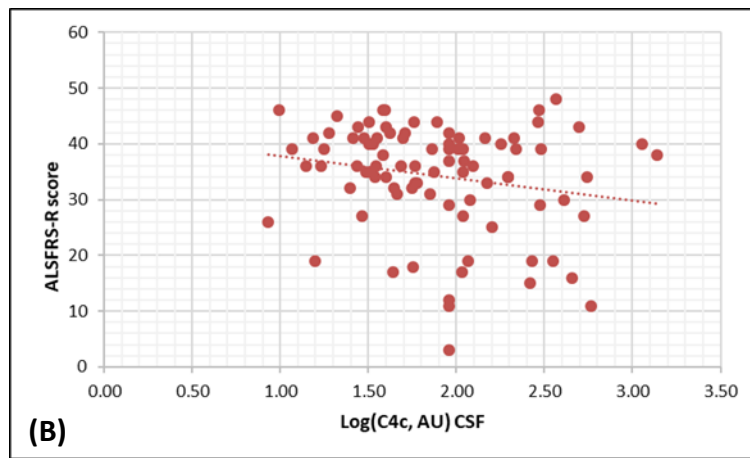
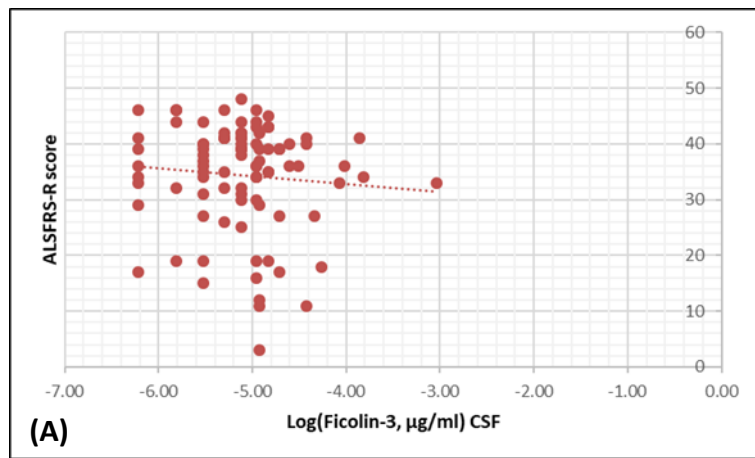
AU: arbitrary unit, CI: confidence interval, CSF: cerebrospinal fluid, EDTA: ethylenediamine tetraacetic acid, HR: hazard ratio, P: p value

Supplementary Figure 1. Univariate regression analysis (Pearson's correlation) of the measured PRMs, complement proteins, and activation products



Supplementary Figure 1 (a-g) shows the ALSFRS-R score in correlation with the measured complement components in plasma/serum. The R^2 and the p-values are found in Table II. ALSFRS-R: Amyotrophic lateral sclerosis functional rating scale – Revised, CSF: Cerebrospinal fluid, Log: Logarithmically transformed, PRM: Pattern recognition molecule, Sqrt: Squareroot transformed

Supplementary Figure 2. Univariate regression analysis (Pearson's correlation) of the measured PRMs, complement proteins, and activation products



Supplementary Figure 2 (A-C) shows the ALSFRS-R score in correlation with the measured complement components in the CSF. The R^2 and the p-values are found in Table II. ALSFRS-R: amyotrophic lateral sclerosis functional rating scale – revised, CSF: cerebrospinal fluid, Log: logarithmically transformed, PRM: pattern recognition molecule