

Supplementary Table S1: ALS patient data.

ALS patients

Pseudonym	Sex	ALS form	Age (years)	Disease duration (months)	ALSFRS-R	ALSFRS-R slope (pt./mo.)	pNfH (pg/ml)	Leukocyte count (cells/ μ l)	Total protein (mg/l)	Albumin quotient	lactate (mmol/l)	Genetics
M3	f	bulbar	73	9	37	1,2	n.a.	1	243	3,8	1,7	
M5	m	spinal	56	3	45	1,0	2589	0	416	6,6	1,7	
M7	m	spinal	49	11	45	0,3	2111	1	582	10,2	1,6	C9orf72
K9	f	spinal	65	4	45	0,8	1577	1	305	5	1,6	
K12	f	spinal	53	8	44	0,5	1258	0	331	5,6	1,7	
K13	m	spinal	53	15	44	0,3	4227	0	309	5,2	1,7	
K14	f	bulbar	57	13	43	0,4	2319	1	363	5,7	1,5	
K15	m	spinal	76	7	41	1,0	10518	1	445	8	1,5	
K16	f	spinal	76	15	33	1,0	4065	2	656	12,6	1,6	
K17	f	spinal	46	48	31	0,4	648	0	310	4,7	1,8	
K19	m	spinal	80	6	35	2,2	7863	2	514	9,5	1,6	
K25	m	spinal	76	7	39	1,3	1520	0	282	5,2	1,5	
K26	m	bulbar	59	10	40	0,8	1725	0	615	10,4	1,8	
K27	m	spinal	56	11	30	1,6	4000	1	458	7,6	1,8	
K29	f	spinal	66	18	42	0,3	1614	1	605	9,9	1,4	
G178	f	spinal	68	32	18	0,9	6786	5	949	17,9	3,6	
Y1	m	spinal	61	18	34	0,8	4119	1	687	12,4	1,4	
M10	m	bulbar	72	18	43	0,3	2181	0	591	11,3	1,4	
M8	m	spinal	72	6	45	0,5	604	0	303	5,1	1,6	
M9	m	spinal	63	72	47	0,0	244	6	403	6,8	1,6	
M11	f	spinal	69	6	45	0,5	1229	1	279	3,6	1,7	
M12	m	bulbar	69	6	44	0,7	3350	1	433	6,2	1,6	
M13	f	spinal	55	10	45	0,3	3561	0	293	4,6	1,5	
M14	f	bulbar	61	32	46	0,1	539	1	380	7,7	1,4	
M15	m	spinal	55	6	36	2,0	5391	1	313	5,6	1,6	
M16	m	bulbar	75	3	40	2,7	6345	0	443	8,7	1,6	
M18	m	spinal	57	35	41	0,2	7057	0	527	9,4	1,7	
M20	f	bulbar	68	30	34	0,5	4294	3	290	5,1	1,8	C9orf72
M21	m	bulbar	49	12	40	0,7	4176	0	395	7,4	1,8	
M22	m	spinal	70	4	45	0,8	2991	2	410	7,3	1,5	
M23	m	spinal	54	7	45	0,4	2298	1	390	6,8	1,5	
M24	m	spinal	60	13	46	0,2	2187	0	316	4,2	1,8	C9orf72
M25	m	spinal	59	41	42	0,1	423	1	367	5,9	1,8	
Mean			63	16	40	0,7	3244	1	430	7	2	
Stand.-Dev.			9,2	15,3	6,2	0,6	2430,3	1,4	152,9	3,1	0,4	
Median			61	11	42	0,5	2454	1,0	395,0	6,8	1,6	
Minimum			46	3	18	0,0	244	0,0	243,0	3,6	1,4	
Maximum			80	72	47	2,7	10518	6,0	949,0	17,9	3,6	

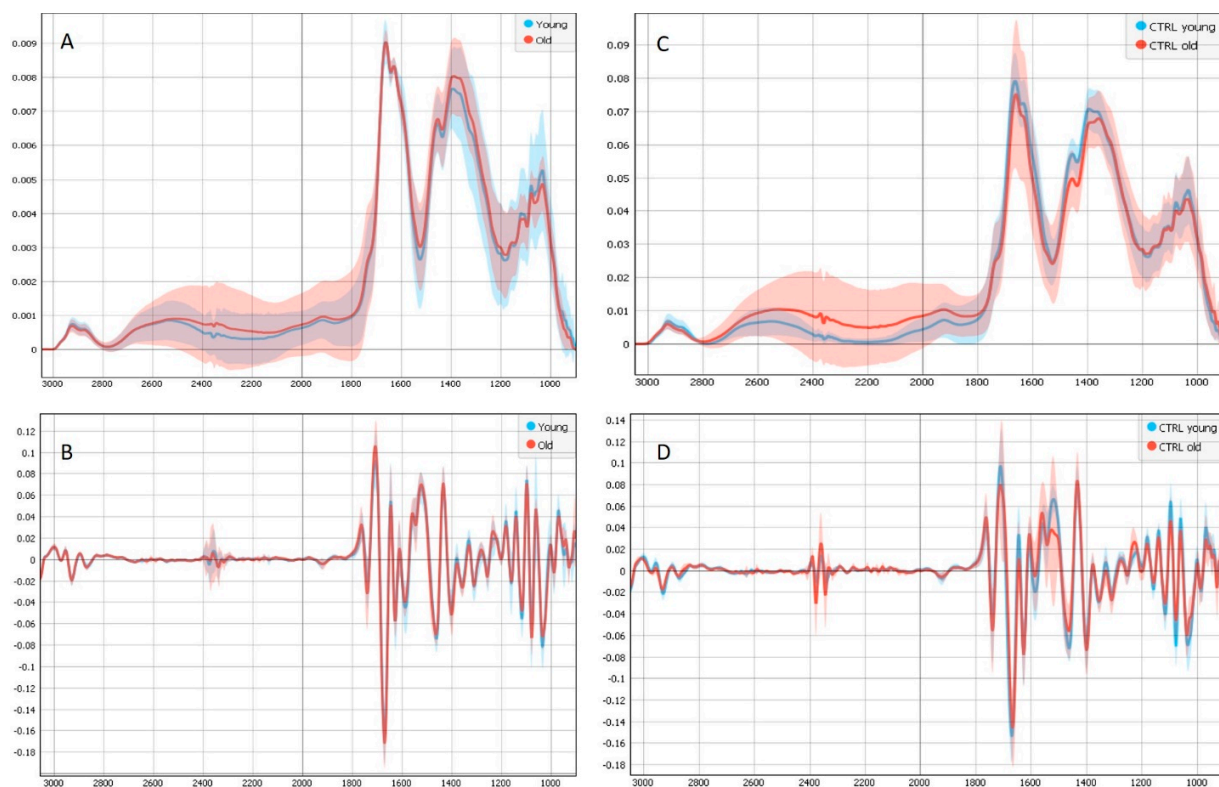
Controls

Pseudonym	Sex	Diagnosis	Age (years)	Leukocyte count (cells/ μ l)	Total protein (mg/l)	Albumin quotient	lactate (mmol/l)
K1	f	IIH	49	2	520	9,4	1,4
K2	f	IIH	57	2	498	8,9	1,5
K4	m	NPH	88	0	386	5,9	2,1
K6	m	BFS	56	0	338	5	1,5
K8	m	Ischemia	78	1	556	9	1,5
K10	f	Dysesthesia	38	1	323	4,8	1,4
K11	f	Dysesthesia	26	0	176	2,3	1,6
K22	f	IIH	46	5	305	5	1,4
K32	f	SMA	22	2	420	6,5	1,2
G237	m	Cereb. Ataxia	80	1	531	10,3	1,8
G242	m	Syncope	80	1	318	5,1	1,8
G243	m	Syncope	75	1	371	5,8	1,6
G245	f	Syncope	54	1	450	7,6	1,6
G248	f	PNP	77	0	349	5,9	1,2
G249	f	PNP	64	0	393	8	1,4
G243	m	Syncope	75	1	371	5,8	1,6
X13	m	ataxia	47	0	438	6	1,3
A80	m	ET	73	1	701	10	1,6
G161	m	dizziness	59	2	440	5,6	1,5
G197	f	SKS	71	0	1142	22,7	1,9
G252	m	CIDP	68	2	456	6,5	2
Q3	f	Rheumatism	52	0	427	8	1,3
Q4	f	SKS	60	1	392	6,7	1,3
P76	m	Tremor	61	2	531	9,2	1,5
Q5	m	vestibular failur	76	1	439	7,3	1,6
X17	m	gait disorder	77	1	713	11,5	1,6
G160	m	headache	51	1	448	7,8	1,4
G190	f	PNP	71	0	532	9	1,3
B30	m	MS	43	1	373	5,2	1,4
G231	m	MS	47	5	715	11,9	1,6
G220	m	PNP	62	1	332	4,7	2,1
G244	m	TGA	57	5	1140	22,4	1,7
Mean			61	1	485	8	2
Stand.-Dev.			16,0	1,4	208,9	4,4	0,2
Median			61	1	439	7	2
Minimum			22	0	176	2	1
Maximum			88	5	1142	23	2

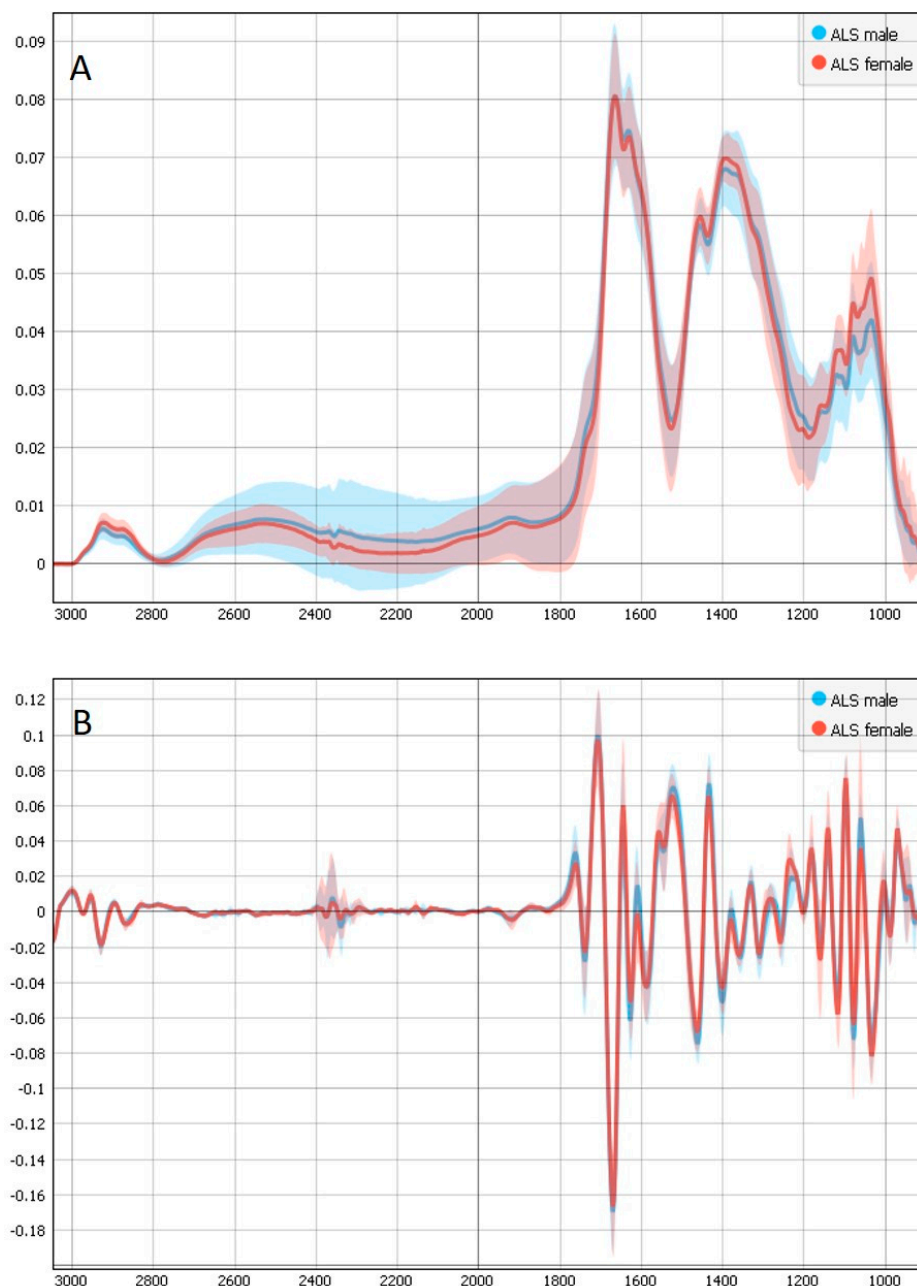


Supplementary Figure S1: Optical images of CSF sample after deposition on a CaF₂ IR window and water evaporation. The color points represent measured points with an aperture of 10 × 10 μm.

This figure represents an overview of the CSF samples prior to the FTIR measurements (composite picture). Six to ten different points per sample were taken for the IR acquisition and the average spectra were taken for further analysis.

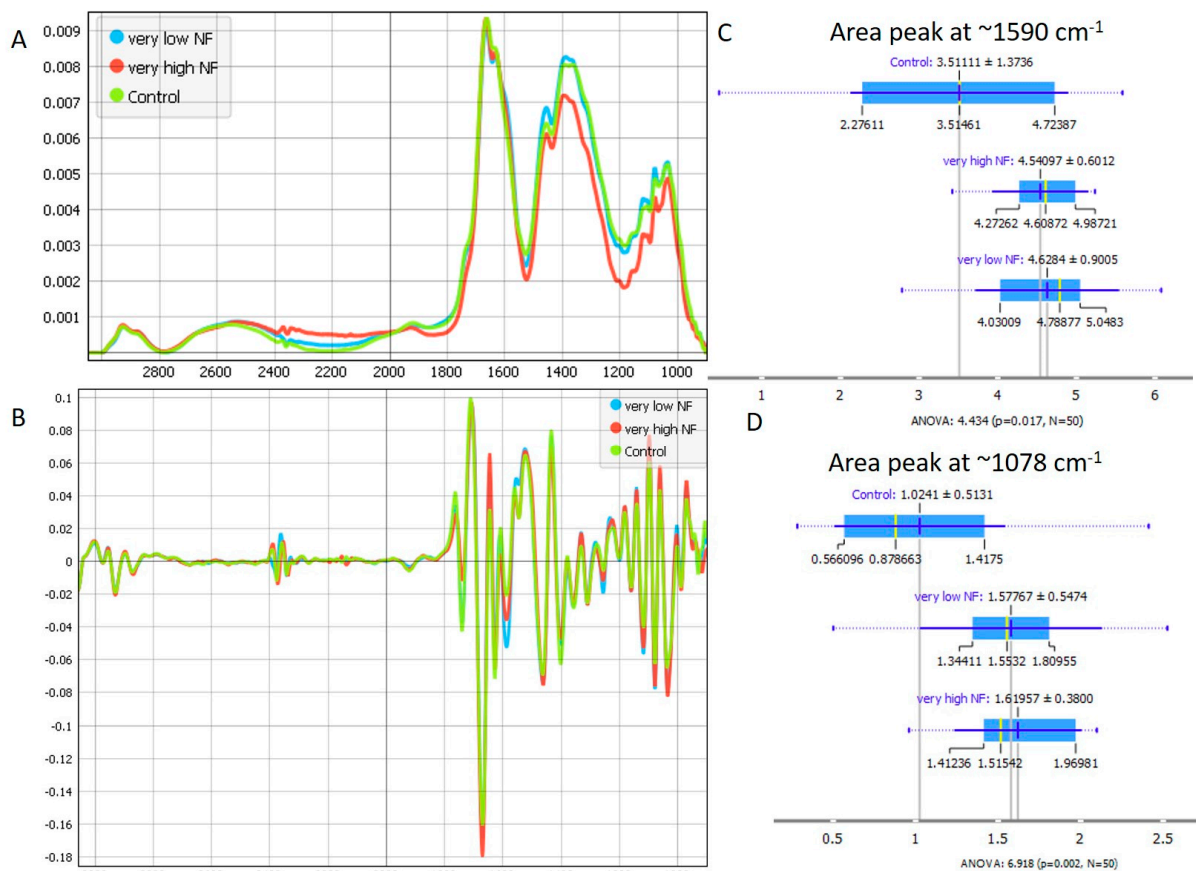


Supplementary Figure S2: Young versus old ALS patients and controls. Whole spectra (A) and the second derivate of the FTIR averaged spectra (B) of samples from young (under 65 years of age; blue) and old ALS patients (over 65 years of age; red) (N=18 and 15, respectively). Whole spectra (C) and the second derivate of the FTIR averaged spectra (D) of samples from young (blue) and old control patients (red) (N=13 and 16, respectively). Control groups (Figure S4C,D) showed more fluctuation of the samples in the fingerprint and lipids part of spectra. However, there are several similarities in the bands between control and ALS group, as for example bands at ~ 1036 , 1120 , 1465 , 1585 cm^{-1} more pronounced in the “young” samples, and assigned to nucleic acids, RNA, CH_2 and CH_3 deformation movements in proteins. In the group of “old” samples, the change at 930 cm^{-1} were marked and this band at is ascribed to the Z-form of DNA. None of these changes was statistically significant.



Supplementary Figure S3: Male vs. female ALS patients. Whole spectra (A) and the second derivate of the FTIR averaged spectra (B) of samples from male (blue) and female ALS patients (red) (N=21 and 12, respectively).

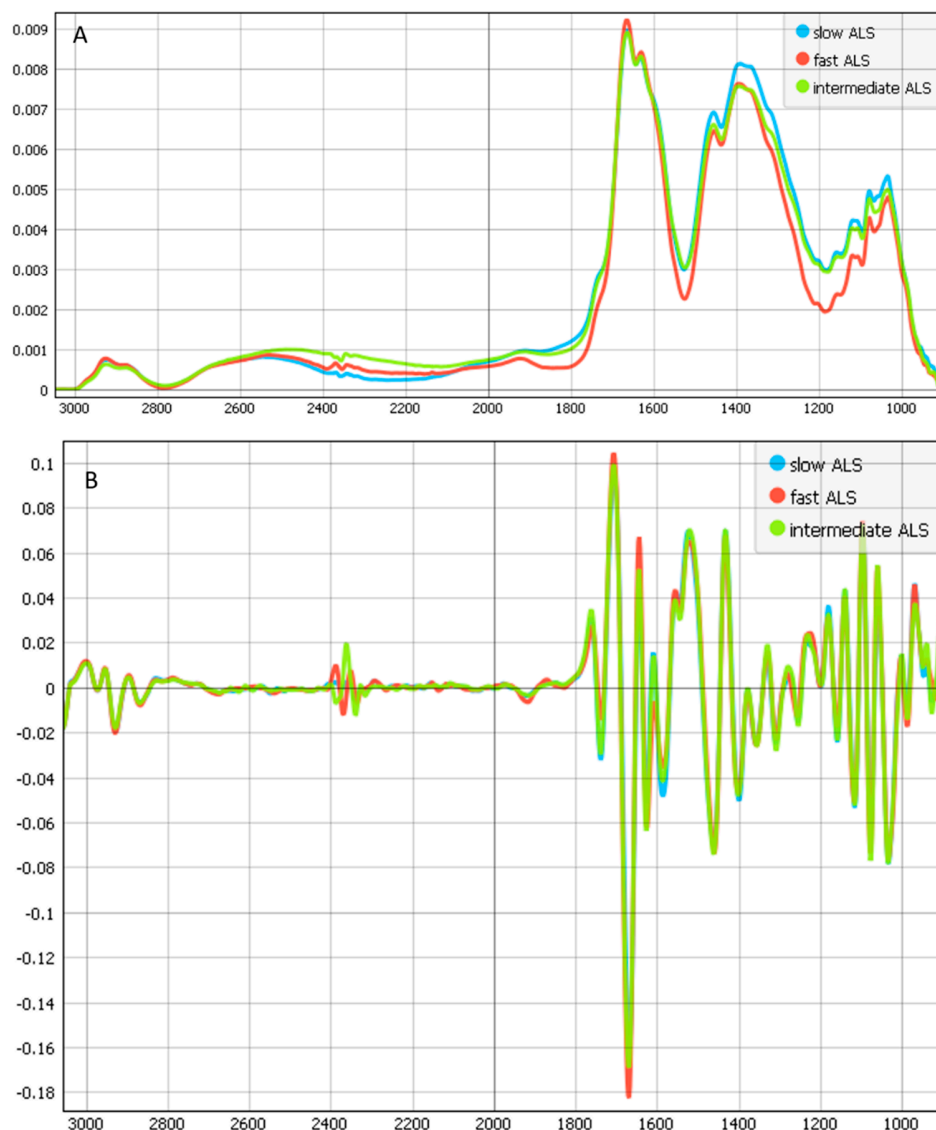
This figure presents the average spectra of the CSF samples from the ALS patients group divided into male and female subgroups. Comparison of spectra did not show significant differences. Nevertheless, there are small differences at 1077 and 1400 cm^{-1} , wavelength corresponding to carbohydrates and carboxyl group, which are more pronounced in the female samples. On the other hand, the male group had more pronounced bands at 1740, 1630, 1400, 1077 cm^{-1} that correspond to carbonyl groups, β -sheet, carboxyl groups and glycogen groups.



Supplementary Figure S4: High vs. low neurofilament vs. control. Whole spectra (A) and the second derivate of the FTIR averaged spectra (B) of samples from patients with relatively low levels of neurofilaments (pNfH <2000 pg/ml; blue) and from patients with relatively high levels of neurofilaments (pNfH >5000 pg/ml; red) and control samples (green). (C) Integrated area under peak at 1590 cm⁻¹. (D). Area under peak at 1078 cm⁻¹. (N=11 for low neurofilaments samples, 7 for high neurofilaments and 32 - control).

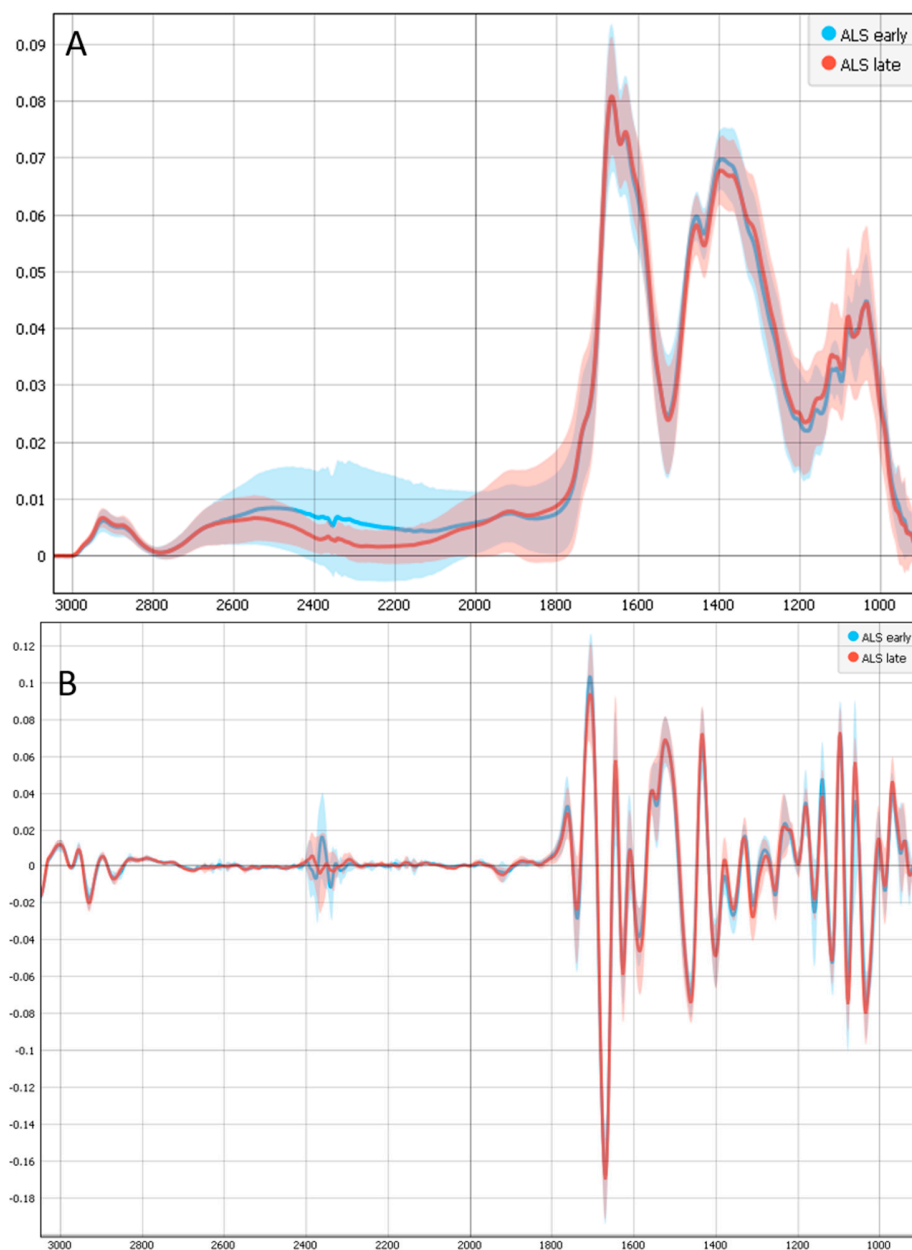
This figure presents the FTIR signature for relatively low (pNfH <2000 pg/ml; LNF) and relatively high (pNfH >5000 pg/ml; HNF) neurofilament levels in the CSF of ALS samples in comparison to control samples. Comparison of spectra show similar shape for control and LNF in comparison with HNF. Significant differences at 1078 cm⁻¹, which correspond to glycogen, and 1587 cm⁻¹ assigned to Glu and Asp and significant lower concentration was found in control samples for both macromolecules, as presented in panel 4C, D, respectively.

There are slight differences at 920, 1034 and 1160 cm⁻¹, wavelength corresponding to Z type of DNA, COH deformation movement in nucleic acids and C-O groups in in proteins and carbohydrates, which are more pronounced in the HNF group. Band at 1740 cm⁻¹, connected to ester groups, were similarly more abundant in the LNF and control in comparison to HNF.



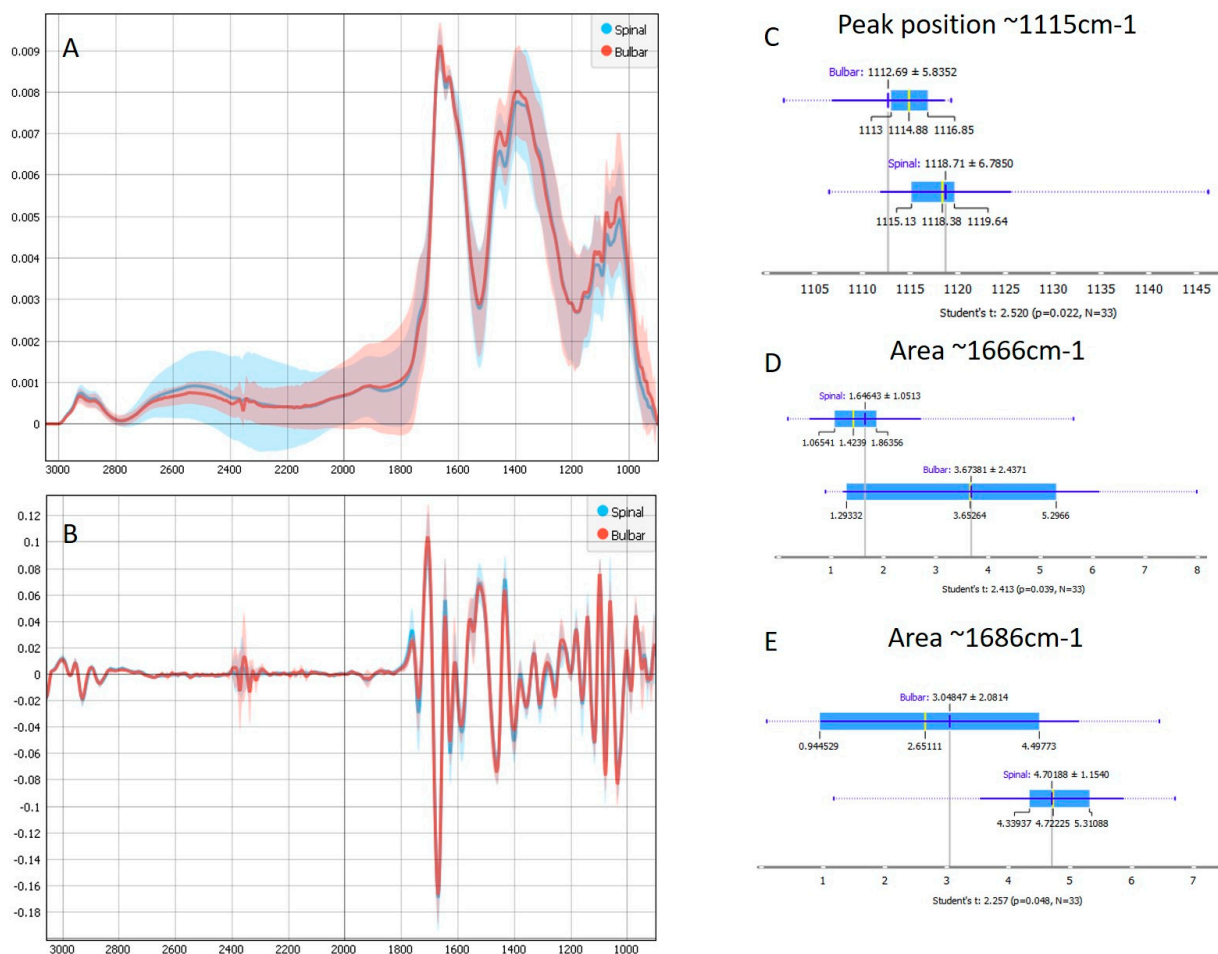
Supplementary Figure S5: Fast vs. slow disease progression (ALSFRS-R slope). Whole spectra (A) and the second derivate of the FTIR averaged spectra (B) of samples from ALS patients with a slow clinical progression (ALSFRS-R Slope <0.5 points per month; blue), intermediate progression (ALSFRS-R Slope between 0.5 and 1 points per month (incl. 0.5); green) and fast progression of disease (ALSFRS-R-Slope >1 point per month (incl. 1); red) (N=9, 10 and 14, respectively).

Figure S6 shows FTIR spectroscopy of ALS samples with fast progression, intermediate and fast progression based on the ALSFRS-R-slope. There are differences observed mostly for fast progression of disease at ~ 920 , 1079, 1107, 1627, and 1670 cm^{-1} , wavelength corresponding to Z-DNA movements, glycogen, RNA conformational changes, and of fibrillary β -sheet and β -turn and loops (proteins conformational changes). On the other hand, the slow ALS progression displays more pronounced bands at 1160 cm^{-1} , 1585 and 1740 cm^{-1} which are connected to glycogen, amino-acids chains as Glu and/or Asp, respectively. However, none of these changes was statistically significant.



Supplementary Figure S6: Early versus late disease stage. Whole spectra (A) and the second derivate of the FTIR averaged spectra (B) of samples from early stage (<11 months after symptom onset) (blue) and late stage (>11months after symptom onset) (red) ALS patients (N=17 and 16, respectively).

This figure presents the signature of the FTIR for early stage (<11 months of disease duration) and late stage (>11 months of disease duration) of the ALS patients. Comparison of spectra did not show significant differences. However, there are slight differences possible to read at 1160, 1360, and 1740 cm⁻¹, wavelength corresponding to and C-O groups in in proteins and carbohydrates, CH₂ and/or CH₃ vibration and carbonyl groups, which are more pronounced in early stage of disease. From the other side, the in the late stage of ALS, there are more pronounced band at 1310 cm⁻¹, which correspond to Amide III, and 1587 cm⁻¹ assigned to Glu and/or Asp.



Supplementary Figure S7: Spinal vs. bulbar ALS. Whole spectra (A) and the second derivate of the FTIR averaged spectra (B) of spinal (blue) and bulbar ALS samples (red) (N=24 and 9, respectively). (C) is showing the significant change in the peak position at ~1115 cm⁻¹. (D): Area under the α -helix structure ~1666 cm⁻¹. E: Area under the β -turn and loops structure ~1686 cm⁻¹.

This figure presents the FTIR spectra of the CSF samples from ALS patients with spinal versus bulbar subtype. The comparison of spectra shows a shift of the position of RNA from 1113 cm⁻¹ for the spinal patients to 1119 cm⁻¹ for the bulbar patients. Regarding the protein structure, there is a significant difference regarding the α -helix band (~1664 cm⁻¹), which is significantly higher in bulbar samples, while the β -turn and loops band (~1680 cm⁻¹) is significantly more pronounced in spinal ALS samples.

Besides, there are slight differences at the bands of ~920, 1034, 1078 and 1120 cm⁻¹, more noticeable in bulbar samples, while in the spinal samples peaks at 1740, 1630, 1590 and 1400 cm⁻¹ are more prominent. These changes corresponding to Z type of DNA, nucleic acids, carbohydrates, and RNA which are more pronounced in bulbar ALS samples. On the other hand, the spinal samples had more evident bands of carbonyl groups, β -sheet, amino acids Glu and/or Asp and carboxyl groups.