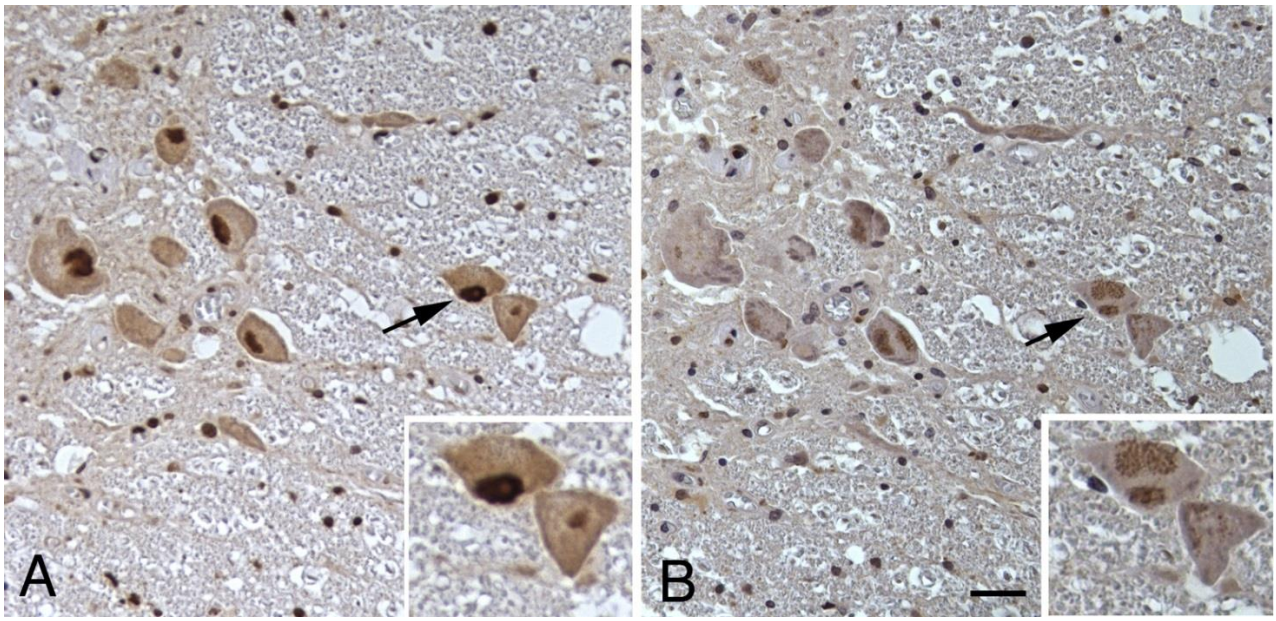
Supplementary Material

Methods: TDP-43/pTDP-43 Immunohistochemistry

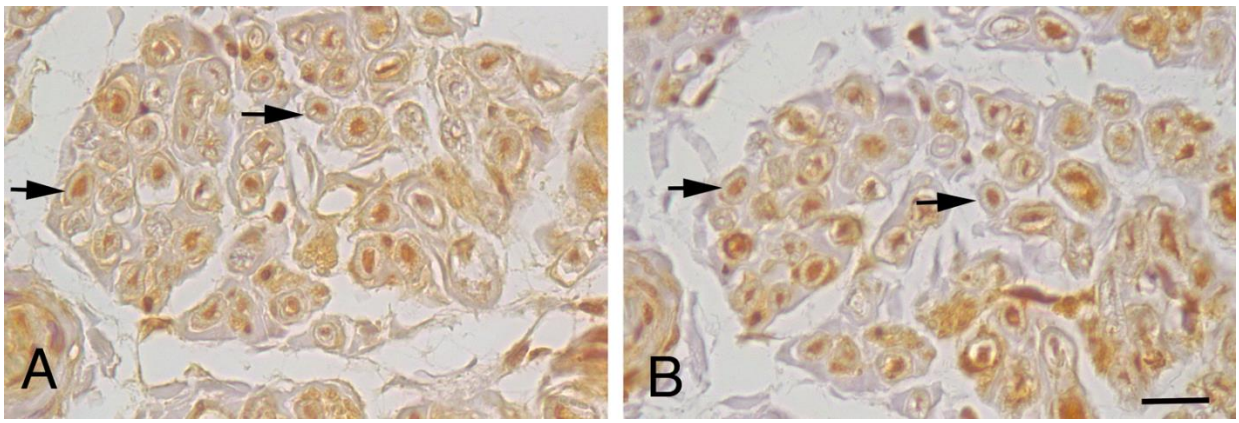
All motor nerve samples were pre-screened for paraffin block availability, cut in three μm-thick sections and stained with haematoxylin and eosin to evaluate tissue quality. Those which passed this first step have been included in the present study. After cut sections were dewaxed in xylene, passed through a graded series of alcohols, and rehydrated in deionised water. Heat-induced epitope retrieval in Tris EDTA pH=9 in a water bath at 97°C for 30 minutes was required for all antibodies. Endogenous peroxidase was exhausted with 10%hydrogen peroxide for 10 min and after that, three washes were performed in 0.05% Tween Tris Buffered Saline solution at pH 7.4 (TBST) for 5 minutes and nonspecific background was blocked with a protein blocking solution, BSA+Tween (2%+0,1%), for 10 minutes at room temperature After three washes, a primary antibody anti-TDP-43 rabbit polyclonal (1:700, Proteintech10782-2-AP) and anti-phospho(pS409/410)-TDP-43 rabbit polyclonal (1:500, Proteintech 22309-1-AP) antibodies diluted in TBST was applied and incubated for 1 h at room temperature. After rinsing the slides for 5 minutes in TBS, biotinylated anti-rabbit were applied for 1 hour and signal amplified with avidin-biotin complex for another hour. The peroxidase reaction was developed for 5 min using diaminobenzidine (Dako K3468) following the manufacturer's instructions and blocked with deionized water. Counterstain with hematoxylin was performed. After dehydration through grading scales of ethanol followed by 20 minutes in xylene, the coverslips were applied on the slides with Eukitt mounting medium and analyzed by light microscopy (Olympus BX51) As positive controls, we used formalin-fixed paraffin embedded post-mortem spinal cord sections obtained from three ALS patients, while one spinal cord section from a non-neurologic subject and nine sural nerves obtained from patients with a diagnosis of neuropathy were used as negative controls. As positive controls, we used formalin-fixed paraffin embedded post-mortem spinal cord sections obtained from three ALS patients (Supplementary Figure 1A and 1B), while spinal cord sections from non-neurologic subjects were used as negative controls. In a subset of ALS and non-ALS samples, pathologic accumulation of pTDP-43 in motor nerves was confirmed by IHC in serial sections stained with anti-ubiquitin mouse monoclonal antibody (1:3000 Millipore MAB1510) (13 vs 13), (Supplementary Figure 2A and 2B).

Supplementary Figure 1. Immunostaining of the spinal cord in ALS.



TDP-43 is seen in nuclei (**A**, arrow); pTDP-43 staining (**B**) shows dense round neuronal cytoplasmic inclusions (arrow). Bar: 50μm.

Supplementary Figure 2. Immunohistochemistry of the motor nerve in ALS.



pTDP-43-immunoreactive axons (**A**) in motor nerve of ALS (arrows). These axons are also ubiquitin-positive (**B**, arrows). Bar: 50μm.

Supplementary Table 1: Histology and TDP/pTDP IHC scores of the study population.

Case #	Final Diagnosis	Histologic Diagnosi	Histopathology					TDP IHC					pTDP IHC				
			FL/DF	AD	AR	My	IC	EN	VN	PN	A	SC	EN	VN	PN	A	SC
1	ALS	pMND	1/2	2	1	0	0	4	4	4	0	1	1	1	1	1	1
2	ALS	pMND	1/1	1	1	0	0	-	-	-	-	-	-	-	-	-	-
3	ALS	pMND	2/1	1	1	0	0	2	1	1	0	0	2	1	1	2	1
4	ALS	pMND	1/1	0	1	0	0	0	3	3	0	0	1	1	1	1	0
5	ALS	pMND	1/1	0	1	0	0	3	4	4	0	0	2	2	2	1	0
6	ALS	pMND	1/1	··	1	0	0	3	3	3	1	1	0	0	0	1	1
7	ALS	pMND	1/1	3	1	0	0	4	4	4	0	0	1	0	1	1	0
8	ALS	pMND	1/1	1	1	0	0	-	-	-	-	-	-	-	-	-	-
9	ALS	pMND	3/1	1	1	0	0	0	2	2	0	0	0	1	2	1	0
10	ALS	pMND	3/1	1	1	0	0	4	4	4	0	0	1	1	1	1	0
11	ALS	pMND	1/1	2	1	0	0	1	4	3	1	1	1	1	1	3	3
12	ALS	pMND	2/1	3	1	0	0	4	4	4	1	1	2	2	2	2	1
13	ALS	pMND	1/2	1	1	0	0	4	4	4	3	0	1	3	3	3	3
14	ALS	pMND	0/0	2	1	0	0	4	4	4	2	1	1	0	0	1	2
15	ALS	pMND	1/1	2	1	0	0	4	4	4	0	0	1	0	0	1	1
16	ALS	pMND	0/0	2	1	0	0	-	-	-	-	-	-	-	-	-	-
17	ALS	pMND	0/0	2	1	0	0	3	3	1	0	0	3	1	2	1	0
18	ALS	pMND	2/1	2	1	0	0	4	4	4	0	0	2	2	0	2	2
19	ALS	pMND	1/1	0	1	0	0	4	4	4	0	1	1	0	0	1	1
20	ALS	pMND	1/1	1	1	0	0	4	4	4	1	1	4	4	4	3	2
21	ALS	pMND	1/1	1	1	0	0	1	0	0	3	3	1	0	0	2	3
22	ALS	pMND	1/1	2	1	0	0	4	4	4	0	1	0	1	0	2	1
23	ALS	pMND	1/1	2	1	0	1	2	3	4	2	2	0	0	0	3	2
24	ALS	pMND	2/1	2	1	0	0	-	-	-	-	-	-	-	-	-	-
25	ALS	pMND	1/1	2	1	0	0	3	4	4	2	1	1	1	2	2	1
26	ALS	pMND	2/1	3	1	0	0	3	2	1	0	0	2	2	1	1	1
27	ALS	pMND	1/2	1	1	0	0	4	4	4	0	1	2	2	2	1	1
28	ALS	pMND	1/1	1	1	0	0	-	-	-	-	-	-	-	-	-	-

29	ALS	pMND	0/0	1	1	0	0	1	3	3	1	1	2	2	1	1	1
30	ALS	pMND	1/1	2	1	0	0	2	4	4	0	1	3	3	3	1	2
31	ALS	pMND	1/1	2	1	0	0	1	1	0	1	0	0	0	1	1	0
32	ALS	pMND	2/1	2	1	0	0	4	4	4	1	0	1	1	3	1	1
33	ALS	pMND	0/0	1	1	0	0	3	4	3	1	2	3	4	4	3	2
34	ALS	pMND	1/2	1	1	0	1	4	4	4	2	1	0	0	2	2	2
35	ALS	pMND	2/1	2	1	0	1	4	4	4	1	1	4	4	4	2	1
36	ALS	pMND	1/2	3	1	0	0	4	4	4	0	2	0	0	0	2	1
37	ALS	pMND	1/1	2	1	0	0	2	3	4	0	0	0	1	0	1	0
38	ALS	pMND	1/1	0	1	0	0	3	3	3	1	1	0	0	0	1	0
39	ALS	pMND	1/2	1	1	0	0	4	4	4	0	1	0	0	0	1	1
40	ALS	pMND	1/1	0	1	0	0	3	4	4	0	0	0	1	1	1	1
41	ALS	pMND	0/0	2	1	0	0	4	4	4	1	2	4	4	4	1	1
42	ALS	pMND	1/2	2	1	0	0	4	4	4	0	1	2	3	2	2	2
43	ALS	pMND	2/1	2	1	0	0	2	4	2	1	1	1	2	1	1	1
44	ALS	pMND	1/1	0	1	0	0	4	4	4	1	1	3	1	0	1	1
45	ALS	pMND	1/1	2	1	0	0	4	4	4	0	1	3	3	3	2	1
46	ALS	pMND	1/1	2	1	0	0	4	4	4	0	1	1	1	1	2	2
47	ALS	pMND	1/1	1	1	0	0	4	4	4	1	1	1	1	1	1	0
48	ALS	pMND	1/1	1	1	0	0	4	4	4	0	0	2	0	1	2	1
49	ALS	pMND	1/1	2	1	0	0	3	3	3	1	1	0	0	0	1	0
50	ALS	pMND	3/1	1	1	0	0	3	4	3	1	1	1	0	0	0	0
51	ALS	pMND	2/1	0	1	0	0	4	4	4	0	1	1	1	1	1	1
52	ALS	pMND	1/1	2	1	0	0	-	-	-	-	-	-	-	-	-	-
53	ALS	pMND	0/0	3	1	0	0	-	-	-	-	-	-	-	-	-	-
54	ALS	pMND	3/1	3	1	0	0	-	-	-	-	-	-	-	-	-	-
55	ALS	pMND	1/1	2	1	0	0	-	-	-	-	-	-	-	-	-	-
56	ALS	pMND	1/1	2	1	0	0	-	-	-	-	-	-	-	-	-	-
57	ALS	ND-N	0/0	1	0	0	0	4	3	3	2	1	0	0	1	2	0
58	ALS	ND-N	0/0	0	0	0	0	0	2	1	2	1	0	1	0	2	0
59	ALS	ND-N	0/0	0	0	0	0	4	4	4	2	2	0	0	0	3	1
60	ALS	ND-N	0/0	0	1	0	0	1	2	1	0	1	0	0	1	1	0

61	ALS	ND-N	0/0	0	0	0	0	2	0	1	0	1	0	1	2	1	1
62	ALS	ND-N	0/0	0	0	0	0	-	-	-	-	-	-	-	-	-	-
63	ALS	ND-N	0/0	0	0	0	0	-	-	-	-	-	-	-	-	-	-
64	ALS	ND-N	0/0	0	0	0	0	4	4	4	0	2	1	0	0	1	1
65	ALS	ND-N	0/0	0	0	0	0	4	4	4	0	0	0	0	0	2	0
66	ALS	ND-N	0/0	0	0	0	0	4	4	4	0	1	0	0	0	2	1
67	ALS	ND-N	0/0	0	0	0	0	-	-	-	-	-	-	-	-	-	-
68	ALS	ND-As	1/1	1	2	0	0	2	1	3	0	0	1	1	1	1	1
69	ALS	ND-As	2/1	1	1	0	2	1	0	3	0	0	0	0	0	1	0
70	ALS	ND-As	1/2	1	2	0	0	4	4	4	1	1	2	4	4	1	1
71	ALS	ND-As	1/1	1	2	0	0	-	-	-	-	-	-	-	-	-	-
79	IF – MMN	MN	0/0	1	3	1	0	2	2	3	0	0	0	0	1	0	0
80	IF – MMN	MN	0/0	0	1	1	0	2	1	2	0	0	1	1	1	0	0
81	IF – MMN	MN	1/2	1	3	0	0	-	-	-	-	-	-	-	-	-	-
75	IF – CMAN	MN	1/1	1	2	1	2	-	-	-	-	-	-	-	-	-	-
82	IF – CIDP	MN	1/2	1	3	1	3	2	1	2	0	0	0	1	0	0	0
74	IF – LSS	MN	0/0	0	3	0	0	-	-	-	-	-	-	-	-	-	-
73	IF – SLE	MN	1/2	1	2	1	2	4	4	4	0	0	4	4	4	0	0
72	IF – NOS	MN	1/1	0	2	1	2	3	3	3	0	0	0	1	1	0	0
76	IF – NOS	MN	1/2	1	2	0	3	-	-	-	-	-	-	-	-	-	-
77	IF – NOS	MN	3/2	1	2	1	1	-	-	-	-	-	-	-	-	-	-
78	IF – NOS	ND-N	0/0	0	0	0	0	-	-	-	-	-	-	-	-	-	-
83	Idiopathic S-M neuropathy	MN	1/2	1	3	0	0	1	1	1	0	0	0	1	0	0	0
84	Idiopathic S-M neuropathy	MN	1/1	0	3	0	0	1	3	3	1	0	0	1	1	0	0
85	Idiopathic S-M neuropathy	MN	1/2	0	3	0	0	4	4	4	0	0	0	0	0	0	0
86	Idiopathic S-M neuropathy	MN	1/2	0	3	0	0	4	4	4	0	0	2	1	1	0	0
87	Idiopathic S-M neuropathy	MN	3/2	1	3	0	0	3	3	3	0	0	2	2	2	0	0
88	Idiopathic S-M neuropathy	ND-N	0/0	0	0	0	0	-	-	-	-	-	-	-	-	-	-

89	Idiopathic S-M neuropathy	ND-As	1/2	0	2	0	0	4	4	4	0	0	0	0	2	0	0
92	Idiopathic M neuropathy	MN	1/2	1	3	1	0	2	0	1	0	1	0	0	0	1	1
93	Idiopathic M neuropathy	MND	2/1	0	1	0	0	3	3	3	1	1	0	1	0	1	0
94	Idiopathic M neuropathy	MND	2/1	1	1	0	0	3	3	3	0	1	0	0	0	2	1
95	Idiopathic M neuropathy	ND-N	0/0	0	0	0	0	3	3	3	0	1	0	0	0	0	1
90	Amyloid neuropathy	MND	1/2	2	1	0	0	-	-	-	-	-	-	-	-	-	-
91	Amyloid neuropathy	ND-N	0/0	0	0	0	0	4	4	4	1	2	1	0	0	2	1
96	Myopathy	ND-N	0/0	0	0	0	0	3	4	3	3	1	0	1	0	3	0
97	Myopathy – IBM	ND-N	0/0	0	1	0	0	3	4	3	0	1	1	1	1	1	0
98	Myopathy – IBM	ND-As	1/1	0	2	0	0	3	4	3	0	0	0	0	0	1	0
99	Spondylotic myelopathy	MND	1/1	0	1	0	0	1	2	2	1	1	0	0	0	0	0
100	Spondylotic myelopathy	ND-N	0/0	0	0	0	0	1	2	2	0	0	0	0	0	0	0
101	Anti-Hu PSMN	MND	3/1	3	1	0	0	2	2	2	0	0	0	0	0	0	0
102	Femoral nerve entrapment	ND-N	0/0	0	0	0	0	2	1	1	0	0	1	1	0	0	0

Histopathologic diagnosis: classified as pathologic motor neuron disease (pMND), motor neuropathy (MN), not diagnostic (ND), the latter further divided as normal (ND-N) or with aspecific findings (ND-As). FL: fiber loss (0 = no signs, 1 = mild, 2 = moderate, 3 = severe); DF: distribution of fiber loss (0 = normal, 1 = focal/multifocal, 2= uniform); AD: Axonal Degeneration (0 = no signs, 1 = mild, 2 = moderate, 3 = severe). AR: clusters of Axonal Regeneration (0 = normal nerve; 1 = low/absent; 2 = moderate; 3 = strong). My = signs of myelin pathology (demyelination or chronic re-myelination) (0 = no signs, 1 = present). IC: inflammatory cells infiltration (0 = no signs, 1 = mild, 2 = moderate, 3 = severe). ⋯: not valuable for the presence of artefacts at pathologic evaluation.

TDP and pTDP IHC scores have been measured semi-quantitatively for each pathologic parameter as follows: 0 = all negative, 1 = number of positive cells < number of negative, 2 = number of positive ≈ number of negative, 3 = number of positive > number of negative, 4 = all positive. EN: endoneurial nuclei. VN: vessel nuclei. PN: perineurial nuclei. A: axons. SC: Schwann cell cytoplasm. -: not done for tissue unavailability.

ALS: amyotrophic lateral sclerosis; IF: Inflammatory neuropathy; MMN: multifocal motor neuropathy; CMAN: chronic motor axonal neuropathy; CIDP = chronic inflammatory demyelinating polyneuropathy; LSS: Lewis-Sumner syndrome; IF – SLE: systemic lupus erythematosus-associated motor neuropathy; IF - NOS: inflammatory neuropathy, not otherwise specified; S-M: sensory-motor; M: motor; IBM: inclusion body myositis; anti-Hu PSMN: anti-Hu paraneoplastic sensorimotor neuronopathy

Supplementary Table 2: Demography and clinical features of the study population.

Case #	Sex	Age at Onset	Disease Duration	Site of Onset	Symmetry	Distal vs proximal	Pyramidal Signs	Sensory Symptoms	Sensory Signs	Anti-GM1 Ab	CSF	ALS-FRS-R
1	F	51	49	LL	A	D	0	1	0	0	1	34
2	M	53	131	UL	S	D	0	0	0			35
3	M	45	32	LL	AS	D	0	1	0	0	1	42
4	F	63	21	LL	AS	P	0	1	1	0	0	42
5	F	29	277	LL	AS	D	0	1	1		0	35
6	M	48	182	UL	S	D	0	0	0			31
7	M	68	10	LL	AS	D	0	1	1	0	1	32
8	M	58	73	LL	S	D	0	0	0	0	0	42
9	M	41	46	LL	AS	P	0	0	0	0	0	37
10	M	44	8	LL	AS	D	0	0	0	0	0	44
11	M	40	10	LL	AS	D	0	0	0	0	0	37
12	M	50	11	LL	AS	P	0	1	0	0	0	44
13	M	68	13	RE	S	-	0	0	0	0	0	17
14	F	32	41	LL	S	D	0	0	0	0	0	34
15	F	69	25	LL	AS	D	0	1	0	0	0	38
16	F	74	4	AX	S	P	0	0	0		0	39
17	M	60	25	LL	AS	D	0	1	1	0	1	46
18	M	52	33	LL	S	D	0	0	0	0	1	27
19	M	21	66	LL	S	P	1	0	0	0		45
20	M	44	31	LL	AS	P	0	0	0	0	0	40
21	M	65	19	UL	AS	P	0	0	0	0		34
22	M	62	38	LL	S	D						
23	M	53	26	UL	AS	D	1	0	0			45
24	F	68	10	LL	AS	D	0	0	0	0	0	29
25	F	56	45	LL	AS	D	0	0	0			40
26 §	M	41	8	LL	S	D						
27	M	48	45	UL	AS	P	0	0	0	0	0	32

28	M	60	28	LL	S	D	0	0	1	0	1	41
29	M	40	10	UL	AS	P	0	0	0			41
30	M	67	6	LL	AS	D	0	0	0	0	0	39
31	M	51	21	LL	AS	D	0	0	0			46
32	M	72	39	LL	AS	D	0	1	1	0	1	42
33	M	45	13	LL	AS	D	1	0	0	2	0	45
34	F	51	45	LL	AS	D	0	0	0	0		45
35	M	29	56	UL	AS	D	0	1	0	0	0	26
36	F	46	68	LL	S	D	0	0	1	0	0	38
37	M	43	7	LL	AS	D	0	0	0	0	0	43
38	M	45	160	LL	AS	D	1	1	1	0		43
39	M	36	22	LL	AS	P	0	0	0	0		46
40	F	16	34	LL	AS	P	0	0	0			43
41	F	56	16	LL	S	D	1	0	0		1	42
42	M	63	4	LL	AS	D	0	0	0			42
43	M	63	47	LL	AS	D	0	0	0	0		46
44	M	66	84	LL	AS	D	0	0	1			42
45	M	66	11	LL	AS	P	1	0	0			39
46	F	58	25	LL	AS	D	0	0	0			35
47	M	76	16	LL	AS	P	0	1	1			32
48	M	17	88	LL	AS	P	1	0	0	0	0	41
49	M	42	23	LL	AS	P	0	0	0			43
50	M	75	14	LL	AS	D	0	0	0	0		44
51	F	68	36	LL	AS	D	0	0	0	0		41
52	M	66	10	UL	S	P	0	1	1	0		39
53	M	60	6	UL	AS	D	1	0	0	0	0	41
54	M	63	29	LL	AS	D	0	0	1	0	0	44
55	M	68	14	LL	AS	D	1	1	1	0	0	43
56	F	28	7	UL	AS	P	1	0	0	0	0	43
57	F	57	44	LL	AS	D	0	0	1	0		42
58	M	50	17	UL	AS	D	0	0	0	0	1	40

59	M	48	15	LL	AS	D	0	1	0	0	1	43
60	F	46	108	LL	S	D						
61	F	64	9	LL	AS	D	0	0	0			
62	F	72	12	LL	AS	D	0	1	1	0	0	42
63	M	42	63	LL	AS	D	0	0	0	0	0	
64	M	43	20	LL	S	D	0	0	0	0		33
65 *	M	70	9	UL	AS	D	0	1	1	0		44
66	M	19	289	LL	AS	D	1	0	0	0	0	42
67	F	66	13	UL	AS	D	0	0	1	0	0	41
68	M	53	13	UL	AS	P	0	0	0	0	0	39
69	F	61	44	LL	AS	D	1	0	0	0		46
70	M	69	17	UL	S	D	1	0	1	1	0	39
71	F	63	28	LL	AS	D	0	0	0	0		41
72	M	35	27	LL	AS	D	0	1	0	0	1	
73	F	42	5	UL	AS	D	0	1	0		1	
74	M	40	244	UL	AS	D	1	0	1	0	0	
75	M	50	5	UL	AS	D	0	0	0	2	1	
76	M	73	40	LL	S	D	0	1	1	0	0	
77	M	51	8	LL	AS	D	0	1	1	0	0	
78	M	35	22	UL	AS	D	0	0	0	0	1	
79	M	33	77	UL	AS	D	0	0	0	0	0	
80	M	44	162	UL	AS	D	0	0	0	0		
81	F	62	109	LL	S	P	0	0	0	0		
82	F	30	112	LL	AS	D	0	1	1	2	0	
83	M	69	12	LL	AS	D	0	0	1	0	0	
84	M	66	18	LL	S	D	0	1	1		0	
85	M	51	131	LL	S	D	0	0	1	0		
86	M	57	201	UL	AS	D	0	0	1	0		
87	F	54	85	CN	S	P	0	1	1	0		
88	M	63	99	LL	S	D	1	0	1	0	0	
89	M	76	2	LL	S	P	0	1	0	0	0	

90	M	57	6	LL	S	D	0	0	0	0	0	
91	M	54	30	LL	AS	D	0	1	1	0	0	
92	F	43	53	LL	S	D	0	0	0		0	
93	M	34	32	LL	AS	P	0	0	0	0	0	
94	M	16	51	LL	S	D	0	0	0	0	0	
95	F	38	17	UL	S	P	0	1	0	0	0	
96	M	39	33	LL	S	P	0	0	0	0		
97	M	69	41	LL	S	P	0	0	0			
98	F	63	88	LL	S	P	1	0	0	0	0	
99	M	56	68	LL	S	D	0	0	1	0		
100	M	63	35	UL	AS	P	1	0	1		0	
101	M	66	12	LL	S	P	0	1	1		1	
102	M	34	22	LL	AS	P		1	1			

Sex: F: female; M: male; Disease duration refers to time from symptom onset to time of biopsy. LL: lower limbs; UL: upper limbs; RE: respiratory. AX: axial CN: cranial nerves. S: symmetrical. AS: asymmetrical; P: proximal; D: distal; 0: absent/normal; 1: present; (For anti GM1 Ab: 0: absent, 1: present, low title, 2: present, high title). CSF: cerebro-spinal fluidALS-FRS-R: amyotrophic lateral sclerosis functional rating scale, revised (retrospectively applied to ALS patients at time of diagnosis. All patients were sporadic. § patient harbouring the *C9orf72* repeat expansion. * patient harbouring the p.Ala382Thr *TARBP* mutation. None of the patients included had evidence of cognitive impairment.

Supplementary Table 3 Multivariate Cox regression survival analysis in ALS

Overall survival	HR	95% CI	*P*	Post-biopsy survival	HR	95% CI	*P*
Age of onset	1.111	1.060-1.165	> 0.001	Age of onset	1.092	1.047-1.140	>0.001
Disease duration at biopsy	0.915	0.882-0.950	> 0.001	Disease duration at biopsy	0.983	0.962-1.005	0.126
R-EEC criteria			0.462	R-EEC criteria			0.414
Pure LMN phenotype	0.948	0.393-2.287	0.905	Pure LMN phenotype	1.293	0.563-2.968	0.545
Possible ALS and higher	2.095	0.629-6.979	0.228	Possible ALS and higher	2.087	0.659-6.611	0.211
Axonal degeneration density			>0.001	Axonal degeneration density			0.011
Mild	7.480	1.812-30.881	0.005	Mild	5.651	1.441-22.159	0.013
Moderate	22.053	5.258-92.485	>0.001	Moderate	9.389	2.518-35.012	0.001
Severe	10.339	2.233-47.873	0.003	Severe	5.340	1.322-21.565	0.019

The analysis revealed that the amount of axonal degeneration (AD) is independently associated with shortened survival in ALS. A stepwise increase in mortality score is observed between low, mild and moderate levels of AD.

Supplementary Table 4: Descriptive frequencies of TDP-43 and pTDP-43 pathologic areas, stratified according to final diagnosis.

		Endoneurial nuclei					**Vessel nuclei**					**Perineurial nuclei**					**Axons**					**SC cytoplasms**				
		ALS		**non-ALS**			**ALS**		**non-ALS**			**ALS**		**non-ALS**			**ALS**		**non-ALS**			**ALS**		**non-ALS**		
		N	**%**	**N**	**%**	***p***	**N**	**%**	**N**	**%**	***p***	**N**	**%**	**N**	**%**	***p***	**N**	**%**	**N**	**%**	***p***	**N**	**%**	**N**	**%**	***p***
TDP pathology		54	94.7%	23	100%	ns	54	94.7%	22	95.7%	ns	55	96.5%	23	100%	ns	26	45.6%	5	21.7%	0.075	38	66.7%	8	34.8%	0.013
Grading	**0**	3	5.3%	0	0%		3	5.3%	1	4.3%		2	3.5%	0	0%		31	54.4%	18	78.3%		19	33.3%	15	65.2%	
	1	6	10.5%	4	17.4%		3	5.3%	4	17.4%		6	10.5%	3	13%		17	29.8%	4	17.4%		31	54.4%	7	30.4%	
	2	7	12.3%	6	26.1%		4	7%	4	17.4%		2	3.5%	5	21.7%		7	12.3%	0	0.0%		6	10.5%	1	4.3%	
	3	10	17.5%	8	34.8%		9	15.8%	6	26.1%		11	19.3%	10	43.5%		2	3.5%	1	4.3%		1	1.8%	0	0.0%	
	4	31	54.4%	5	21.7%		38	66.7%	8	34.8%		36	63.2%	5	21.7%		0	0%	0	0%		0	0%	0	0%	
pTDP pathology		37	64.9%	7	30.4%	0.007	36	61.4%	12	52.2%	ns	36	63.2%	9	39.1%	ns	56	98.2%	7	30.4%	**<0.0001**	40	70.2%	4	17.4%	**<0.0001**
Grading	**0**	20	35.1%	16	69.6%		21	36.8%	11	47.8%		21	36.8%	14	60.9%		1	1.8%	16	69.6%		17	29.8%	19	82.6%	
	1	19	33.3%	4	17.4%		20	35.1%	10	43.5%		18	31.6%	6	26.1%		33	57.9%	4	17.4%		28	49.1%	4	17.4%	
	2	10	17.5%	2	8.7%		7	12.3%	1	4.3%		9	15.8%	2	8.7%		17	29.8%	2	8.7%		9	15.8%	0	0.0%	
	3	5	8.8%	0	0%		4	7%	0	0%		4	7%	0	0%		6	10.5%	1	4.3%		3	5.3%	0	0.0%	
	4	3	5.3%	1	4.3%		5	8.8%	1	4.3%		5	8.8%	1	4.3%		0	0%	0	0%		0	0%	0	0%	

Data are expressed in numbers (%). Statistical inference between the two disease groups was tested for presence or absence of protein reactivity in the examined areas. Fisher exact test was applied, with a Bonferroni corrected significance threshold of $p<0.005$ to account for multiple comparisons; areas significantly associated with ALS are indicated in bold.

Supplementary Table 5: Diagnostic parameters of R-EEC, histopathologic patterns and peripheral pTDP-43 pathology for ALS.

	Sensitivity	Specificity	Positive LR	Negative LR	DOR	Accuracy
R-EEC	11.3% (5.8-20.7)	100% (88.9 - 100)	NA	0.9 (0.8 – 1. 0)	NA	38.2 % (28.8-48.4)
Morphology patterns (n=102)						
Pathologic MND	78.9% (68.0 – 86.8)	83.9% (67.4 – 92.9)	4.9 (3.3 – 7.3)	0.2 (0.2 – 0.3)	19.4 (6.4 – 59.1)	80.39 (71.65-86.93)
Pathologic MN	51.6% (34.8 – 68.04)	100% (94.9 - 100)	NA	0.5 (0.4 – 0.5)	NA	85.29 (77.15-90.88)
IHC patterns (n=80)						
pTDP-43 pathology[a]	98.2% (90.7 – 99.7)	65.2% (44.9 – 81.2)	2.8 (2.2 – 3.6)	0.03 (0.00 – 0.20)	105 (12.2 – 906.5)	88.75 (79.9-93.8)
Axonal	98.2% (90.7 – 99.7)	69.6% (49.1 – 84.4)	3.2 (2.4 – 4.3)	0.02 (0.003 – 0.19)	128 (14.6 - 1119)	90% (81.5-94.8)
SC cytoplasm	70.2% (57.3 – 80.5)	82.6% (62.9 – 93.0)	4.03 (2.4 – 6.7)	0.4 (0.32 – 0.41)	11.2 (3.3 – 37.8)	73.75% (63.18-82.14)
Morphology and IHC (n=80)						
Pathologic MND + Ax pTDP-43	78.9% (66.7 – 87.5)	91.3% (73.2 – 97.6)	9.1 (3.4 – 24.5)	0.23 (0.19 – 0.27)	39.4 (8.1 – 191.9)	82.5% (72.74 – 89.28)
Pathologic MND + SC pTDP-43	59.6% (46.7 – 71.4)	95.7% (79.0 – 99.2)	13.7 (1.9 – 101.3)	0.42 (0.39 – 0.46)	32.5 (4.1 – 258.4)	70% (59.23 – 78.94)

Sensitivity and specificity are expressed in percentage (95% CI). LR: likelihood ratios; DOR: diagnostic odds ratio. Positive/Negative LR and DOR are expressed as ratios (95% CI). [a] pTDP-43 pathology: either axonal of SC cytoplasm positivity. NA: not applicable.

We calculated the following diagnostic parameters were from 2x2 contingency tables: sensitivity, specificity, likelihood ratios (LR), diagnostic odds ratios (DOR) and accuracy. Histological features satisfying criteria for pMND appeared to be both sensitive (Se: 78.9%) and specific (Sp: 83.9%) for ALS detection (eTable 4 in the Supplement). Diagnosis of pMN showed very high specificity (Sp: 100%), but only a moderate sensitivity (Se: 51.6%). The detection of pTDP-43-positive axons provided the highest sensitivity for the detection of ALS (Se: 98.2%), associated with a strong negative Likelihood ratio (LR: 0.02), and moderate specificity (Sp: 69.6%), while pTDP-43 aggregates in SCs cytoplasms showed moderate sensitivity (Se: 70.2%), but better specificity (Sp: 82.6%). The combination of both morphologic and pTDP-43 pathology yielded an increase in specificity compared to pMND alone, ranging from 91·3%, when combining pMND and axonal pTDP-43, up to 95.7% when pMND and pTDP-43 in SCs cytoplasms were both detected, despite a reduction in sensitivity (Sp: 78.9 and 59.6%, respectively).