Elevated plasma p-tau181 levels unrelated to Alzheimer’s disease pathology in amyotrophic lateral sclerosis

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ABSTRACT
Background Phosphorylated-tau181 (p-tau181), a specific marker of Alzheimer’s disease (AD) pathology, was found elevated in plasma but not in cerebrospinal fluid (CSF) of patients with amyotrophic lateral sclerosis (ALS). We expanded these findings in a larger patient cohort, exploring clinical/electrophysiological associations, prognostic value and longitudinal trajectories of the biomarker.

Methods We obtained baseline plasma samples from 148 ALS, 12 spinal muscular atrophy (SMA), and 88 AD patients, and 60 healthy controls. Baseline CSF and longitudinal plasma samples were from 130 and 39 patients with ALS. CSF AD markers were measured with the Lumipulse platform, and plasma p-tau181 with SiMoA.

Results Patients with ALS showed higher plasma p-tau181 levels than controls (p<0.001) and lower than AD participants (p=0.02). SMA patients had higher levels than controls (p=0.03). In patients with ALS, CSF p-tau and plasma p-tau181 did not correlate (p=0.37). Plasma p-tau181 significantly increased with the number of regions showing clinical/neurophysiological lower motor neurons (LMN) signs (p=0.007) and correlated with the degree of denervation in the lumbosacral area (r=0.51, p<0.0001). Plasma p-tau181 levels were higher in classic and LMN-predominant than in bulbar phenotype (p=0.004 and p=0.006). Multivariate Cox regression confirmed plasma p-tau181 as an independent prognostic factor in ALS (HR 1.90, 95% CI 1.25 to 2.90, p=0.003). Longitudinal analysis showed a significant rise in plasma p-tau181 values over time, especially in fast progressors.

Conclusions Plasma p-tau181 is elevated in patients with ALS, independently from CSF levels, and is firmly associated with LMN dysfunction. The finding indicates that p-tau181 of putative peripheral origin might represent a confounding factor in using plasma p-tau181 for AD pathology screening, which deserves further investigation.

WHAT IS ALREADY KNOWN ON THIS TOPIC
⇒ A recent study showed that p-tau181 levels in plasma, but not in cerebrospinal fluid, are elevated in patients with ALS and correlate with lower motor neuron (LMN) dysfunction, although only clinically assessed.

WHAT THIS STUDY ADDS
⇒ In the most significant cohort studied to date, we confirmed that plasma p-tau181 is higher in patients with ALS than in controls and show that it has a solid prognostic value and increases along the disease course, especially in patients with a faster disease progression. The biomarker is related to the spatial extent of clinical/neurophysiological LMN dysfunction and the denervation degree in the lumbosacral region assessed through neurophysiological studies.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY
⇒ Plasma p-tau181 is a biomarker of LMN dysfunction in ALS. The finding also implies that the marker is not fully specific to the Alzheimer’s Disease (AD) pathological process. Future use of plasma p-tau181 as a screening tool for AD in the general population should consider the peripheral source of p-tau as a possible confounding factor.

INTRODUCTION
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterised by the progressive degeneration of motor neurons in the brain and spinal cord, leading to widespread muscle wasting and weakness.1 Clinically, ALS is a highly heterogeneous disease, ranging from classical to rarer phenotypes with the predominant involvement of upper motor neurons (UMN) or lower motor neurons (LMN), potentially challenging the diagnostic process. The possible association of cognitive impairment, sometimes leading to full-blown frontotemporal dementia,2 further broadens the clinical spectrum of ALS. Neuropathologically, ALS belongs to the TDP-43 proteinopathies characterised by TDP-43-enriched inclusions in affected neurons.3,4

In recent years, innovative biofluid biomarkers have contributed to remarkable progress in neurodegenerative diseases, allowing earlier and more accurate diagnostic and prognostic evaluations and a deeper understanding of pathophysiological mechanisms. Biomarkers of Alzheimer’s disease...
(AD) pathophysiology, namely amyloid-beta, phospho-tau (p-tau) and total tau (t-tau), and those detecting neuroaxonal degeneration, as neurofilament light chain (NFL), have provided the most substantial impact. Moreover, reliable assays that can detect p-tau and NFL in blood have become available, paving the way for more widespread use of these biomarkers in clinical practice. In particular, the measurement of blood p-tau is increasingly considered a realistic, cost-effective and non-invasive assay that will help the diagnostic process for patients with cognitive decline. Nevertheless, whether plasma measures of these biomarkers exclusively reflect their cerebrospinal fluid (CSF) concentration or are also influenced by peripheral sources remains to be fully explored.

In motor neuron diseases, NFL levels in CSF and plasma have been shown to accurately distinguish patients with ALS from their mimics, correlate with disease severity and predict survival. Tau protein isoforms in biofluids have also been investigated in ALS, either as a marker of neurodegeneration (t-tau) or as a follow-up of studies reporting a small amount of p-tau deposition in the brain and the spinal cord of patients with ALS with cognitive dysfunction and, to a lesser extent, in those with pure motor ALS. ALS subjects showed significantly higher CSF t-tau levels than controls, probably reflecting unspecific massive neurodegeneration, whereas inconclusive results were obtained for CSF p-tau. Unexpectedly, a recent study by Cousins et al showed that patients with ALS exhibit significantly increased levels of plasma p-tau phosphorylated at residue 181 (p-tau181) compared with controls. Intriguingly, the authors found that plasma p-tau181 levels do not correlate with CSF p-tau181 levels and AD postmortem neuropathological changes. Moreover, they demonstrated a significant association between plasma p-tau181 levels and the degree of LMN loss in the cervical, thoracic and lumbar sacral districts, supporting a peripheral origin of the plasma p-tau181 elevation.

Given the potential relevance of the finding also for the AD fields, given that plasma p-tau isoforms, including p-tau181, are being increasingly proposed as a screening marker of AD pathology, we aimed to expand the current data on the plasma p-tau181 levels in patients with ALS. Furthermore, we explored for the first time the association of the biomarker with electrophysiological variables and survival and studied the longitudinal trajectory of the biomarker during the disease course. Finally, we extended the analysis of plasma p-tau181 in patients with a different form of motor neuron disease, namely, spinal muscular atrophy (SMA).

METHODS
Inclusion criteria and clinical assessment
Our cohort comprised 148 patients with a clinical diagnosis of ALS according to the Revised El Escorial criteria, evaluated at the Institute of Neurological Sciences of Bologna between September 2014 and July 2022. Among them, 130 had samples of both CSF and plasma available and a negative amyloid status according to the A/T/N classification. We also included 18 patients with ALS with only plasma samples available because their age at sampling (less than 60, median 55.5, IQR 47.25–57) made a concomitant AD pathology unlikely. Finally, we included 20 ALS patients with CSF evidence of underlying amyloid co-pathology (A+), 12 SMA patients, 88 patients with AD and 60 healthy controls.

All SMA patients (7 SMA type 2 and 5 SMA type 3) had a genetically confirmed diagnosis and were treatment-naive. Patients with AD fulfilled the criteria for ‘probable AD dementia with evidence of the AD pathophysiological process’ according to the 2011 NIADA criteria.

For ALS patients, the following clinical data were collected at baseline: age at onset, sex, disease duration (time elapsed between the first referred symptom and sampling), type of onset, clinical phenotype, ALS Functional Rating Scale-Revised (ALSFRS-R) score, Medical Research Council (MRC) scale of 0–5 (calculated as the sum of 10 muscles for each side score/20; score 0–5 points), forced vital capacity (FVC), body mass index (BMI), creatinine levels, King’s, Milan-Torino (MiTos) and Fine’til 9 (FT9) clinical stages. Patients were stratified according to the validated clinical classification in classic, bulbar, respiratory, UMN-predominant (PUMN), primary lateral sclerosis (PLS), flail arm syndrome, flail leg syndrome and progressive muscular atrophy (PMA). However, to allow comparisons with sufficient statistical power, we grouped them in main categories: classic (including respiratory), bulbar, PUMN (ie, PUMN and PLS) and LMN-predominant (PLMN, including flail arm/leg and PMA).

Details on cognitive function assessment in patients with ALS are provided in online supplemental materials. One hundred and forty-two (96%) patients underwent genetic screening for the most frequent ALS-associated genes (ie, SOD1, FUS, TARDBP and the repeats-expansion of C9Orf72), (online supplemental materials). UMN involvement was evaluated by the number of regions (bulbar, cervical and lumbar sacral region) showing UMN signs at clinical examination. In contrast, we used clinical and electromyographic (EMG) assessments according to the Awaji criteria to define the extent of LMN involvement.

To further investigate the correlation between plasma p-tau181 levels and LMN dysfunction, we assigned to each patient with available EMG data (n=119) a denervation score (DS), as reported. Briefly, in the affected muscle with the highest denervation activity (DR, sharp waves or fibrillation) in each region (bulbar, cervical or lumbar sacral), we derived a numerical score (0–10) based on the number of sites per muscle showing DR, with each muscle explored in 10 sites.

The disease progression rate at the baseline visit (b-DPR) was calculated as follows: (48–ALSFRS-R score at the time of sampling/months elapsed between disease onset and sampling). Accordingly, patients were divided into slow (b-DPR<0.5), intermediate (b-DPR 0.5–1) and fast progressors (b-DPR>1).

Thirty-nine of the 148 patients with ALS had plasma samples from two or more follow-up visits. Repeated sampling was performed at non-standardised time points after the diagnostic assessment. In detail, 15 patients were sampled twice, 12 three times, and nine and three patients had samples from 4 and 5 visits, respectively. The median follow-up was 13 months (IQR 7–22). For these patients, we calculated the longitudinal disease progression rate (l-DPR) as the change in the ALSFRS-R between the last and the baseline visits divided by the number of months between the visits. Accordingly, patients with ALS were further classified into fast progressors (l-DPR>1), intermediate progressors (l-DPR 0.5–1) and slow progressors (l-DPR<0.5).

CSF and plasma analyses
EDTA plasma samples were collected, aliquoted and stored at −80°C according to standard procedures. CSF samples were obtained by lumbar puncture (LP), centrifuged in case of blood contamination, divided into aliquots and stored in polypropylene tubes at −80°C until analysis.

Plasma p-tau181 and NFL levels in all participants, and CSF NFL values in patients with ALS, were determined with the
Single molecule array (Simoa) technology on a SR-X instrument using commercially available kits (Quanterix, Billerica, Massachusetts, USA). The mean intra-assay and interassay coefficients of variation (CVs) were below 15% for both biomarkers. CSF NfL in patients with AD was quantified by a validated commercial ELISA assay (NIL ELISA kit, IBL, Hamburg, Germany). CSF t-tau, p-tau181, Aβ42 and Aβ40 were measured by automated chemiluminescent enzyme immunoassay on the Lumipulse G6000H1 platform (Fujirebio, Gent, Belgium). The mean intra-assay and interassay CVs for these markers were <8%. The Aβ42/Aβ40 was calculated as described. Pathological values for the AD core markers were determined according to in-house validated cutoffs. Specifically, a CSF Aβ42/Aβ40×10 ratio <0.68 was considered supportive of amyloid deposition (ie, A+ according to the ATN classification).

Statistical analyses
Statistical analysis was performed using Stata SE V.14.2 (StataCorp) and GraphPad Prism V.7 (GraphPad Software, La Jolla, California, USA) software.

For continuous variables, the Kruskal-Wallis test (followed by Dunn-Bonferroni post hoc test) or the one-way analysis of variance (followed by Tukey’s post hoc test) were used for multiple group comparisons. The χ² test was applied for categorical variables.

For the cross-sectional analysis, Spearman’s r coefficient was used to test the correlation between plasma p-tau181 and clinical/neuropsychological variables. Furthermore, the association between plasma p-tau181 and the degree of UMN and/or LMN involvement was assessed using univariate and multivariate linear regression models with the log-transformed plasma p-tau181 values as dependent variables and the extent of (1) UMN involvement, (2) LMN involvement, (3) UMN and LMN involvement as independent variables. In the multivariable models, we adjusted for age at sampling, sex, genetic status, presence of FTD, b-RS-R scale, ALS phenotype, type of onset, MRC and King's scores. The results are presented as β coefficients and 95% CI.

For the prognostic analysis, the cumulative time-dependent probability of death was calculated by the Kaplan-Meier estimate. The time of entry into the analysis was the date of the first sampling, and the time of the endpoint was the date of death/tracheostomy or the date of the last follow-up information, whichever came first. We performed univariate and multivariate Cox regression models to study prognostic factors in ALS. The multivariate Cox regression analysis was adjusted for age at onset, type of onset, ALSFRS-R score, presence of FTD, b-DPR and King’s score. The results are presented as hazard ratios (HRs) and 95% CI. The assumption of proportional hazard was assessed by Schoenfeld residuals. Differences were considered significant at p<0.05.

For the longitudinal analysis, a linear mixed effect modeling analysis with a random slope and random intercept was performed to evaluate the rate of change over the time of plasma p-tau181 in the patients with ALS stratified into fast, intermediate and slow progressors, according to both basal and longitudinal DPR. The results are presented as β coefficients and 95% CI.

RESULTS
Demographic data and distribution of plasma p-tau181 values across the diagnostic groups
Demographic and clinical data of the studied population are shown in tables 1 and 2.

Post hoc analysis showed no significant difference in the age at sampling between ALS patients and controls (p>0.99).

Plasma p-tau181 levels significantly differed across diagnostic categories (table 1 and figure 1). Post hoc analysis showed that patients with ALS and AD had substantially higher p-tau181 levels than controls (p<0.001), with significantly lower levels in ALS compared with AD (p=0.02). SMA patients also showed significantly higher p-tau181 levels than controls (p=0.03), in line with ALS participants (p=0.42). Of note, patients with ALS (main group) had significantly lower plasma p-tau181 values than A+ALS patients (p=0.005). In contrast, patients with ALS showed significantly lower CSF p-tau values than AD participants (p<0.001). The distribution

| Table 1 Demographic variables and biomarker values in the study population across the different diagnostic categories |
|----------------------------------|----------------|----------------|----------------|-----------------|---------------|
|                                 | ALS n=148      | ALS A+n=20     | AD n=88        | SMA n=12        | Controls n=60  |
| Female, N (%)                   | 54 (36.5)      | 9 (45.0)       | 53 (60.2)      | 6 (50)          | 26 (43.3)      |
| Age at plasma sampling, years   | 62 (51–69)     | 74.5 (70.0–81.5)| 67 (61–73.5)   | 35.5 (25.5–48.5)| 60.5 (58.2–63.0)|<0.0001* |
| Plasma p-tau 181†              | 2.47 (1.40–4.29)| 4.39 (2.68–6.31)| 3.26 (2.46–4.30)| 1.62 (0.95–2.69)| 1.04 (0.78–1.26)|<0.0001† |
| Plasma NfL†                    | 73.5 (42.8–116.1)| 58.8 (38.5–103.0)| 21.1 (16.8–26.4)| –               | 10.1 (8.5–14.6)|<0.0001** |
| CSF p-tau181†                  | 33 (26.2–42.6) | 58.1 (43.7–80.1)| 109 (82–159)   | –               | –             |<0.0001†† |
| CSF NfL†                       | 6307 (3250–12011) | 4324 (2329–6390) | 1076 (862.5–1488)| –              | –             |<0.0001*** |
| ** Post hoc test: ALS vs ALS A+, ALS A+ vs SMA, ALS A+ vs controls and AD vs SMA p<0.0001, ALS vs AD and ALS vs SMA p=0.002, ALS A+ vs AD and AD vs controls p=0.01, SMA vs controls p=0.008; †Data are expressed as median (IQR). |
| ††Data are available only in 144 patients. |
| ‡‡Data are available only in 91 patients. |
| * Post hoc test: ALS vs ALS A+ p=0.005, ALS vs AD p=0.02, ALS vs controls, ALS A+ vs controls and AD vs controls p<0.0001, ALS A+ vs SMA p=0.002, AD vs SMA p=0.02, SMA vs controls p=0.03. |
| §§Data are available only in 117 patients. |
| ¶¶Data are available only in 18 patients. |
| **Post hoc test: ALS vs AD and ALS A+ vs AD, p<0.0001. |

AD, Alzheimer’s disease; ALS, amyotrophic lateral sclerosis; ANOVA, analysis of variance; SMA, spinal muscular atrophy.
of CSF core AD biomarkers is shown in online supplemental table 1.

Association between biomarkers and clinical variables in patients with ALS

CSF p-tau181 and plasma p-tau181 did not correlate at baseline (r=0.08, p=0.37). In contrast, cNFL and pNFL values strongly correlated at first LP (r=0.79, p<0.001). Plasma p-tau181 was not associated with pNFL (r=0.03, p=0.69) or cNFL (r=−0.03, p=0.72). The lack of correlation between plasma p-tau181 and NFL levels extended to the ALS A- (r=0.01, p=0.87) and ALS A+ (r=0.28, p=0.23) subgroups.

Plasma p-tau181 levels were weakly correlated with age at sample collection (r=0.25, p=0.02) and showed significantly higher values in males than females (median 2.77, IQR (1.55–4.82) vs 1.89 (1.18–2.92), p=0.009).

We also found a weak correlation between plasma p-tau181 and ALSFRS-R (r=−0.21, p=0.01) and MRC (r=−0.37, p<0.0001), while there were no associations with BMI (p=0.098), King’s stage (p=0.06), MiToS (p=0.33), FT9 (r=0.16, p=0.052), creatinine values (p=0.46), CVF (p=0.22) or b-DPR (p=0.78). The disease duration correlated weakly with only a trend of significance (r=0.16, p=0.05).

Plasma p-tau181 levels significantly differed across clinical onset types (p=0.005), and post hoc analysis revealed significantly higher levels in spinal than in bulbar onset (p=0.005), accordingly, plasma p-tau181 levels significantly differed across ALS phenotypes (p=0.004), with post hoc analysis revealing considerably higher levels in classic than bulbar ALS (p=0.004) and in PLMN compared with bulbar ALS (p=0.006, table 3).

Plasma p-tau181 levels were significantly lower in FTD-ALS than in pure motor ALS patients (2.7 (1.73–4.68) vs 1.33 (1.04–1.59), p=0.0001).

Finally, plasma p-tau181 levels were significantly influenced by genetic status (p=0.007), with increased levels in patients carrying mutations in SOD1 compared with C9ORF72 (p=0.04) and TARDBP-mutated patients (p=0.04) (table 3).

Association between plasma p-tau181 and the extent of UMN and/or LMN degeneration in patients with ALS

Plasma p-tau181 levels were not associated with the number of body regions displaying UMN signs (p=0.10) or the number of districts showing both UMN and LMN signs (p=0.98). Conversely, there was a weak association with the number of body regions displaying LMN signs (r=0.28, p=0.0008). Accordingly, p-tau181 levels significantly increased with the increasing number of regions affected by isolated LMN signs (p=0.007), but not with the number of areas displaying isolated UMN signs or UMN and LMN signs (p=0.20 and 0.92, respectively) (table 4).

After adjustment for covariates (ie, age, sex, genetic status, presence of FTD, ALSFRS-R scale, ALS phenotype, type of onset, MRC score and King’s stage), the association between plasma p-tau181 levels and the number of regions displaying LMN signs remained statistically significant (three areas vs one region: β=−0.46, 95% CI −0.89 to 0.03, p=0.036, online supplemental table 2). Notably, the association was still significant after adding plasma NFL levels in the multivariate model (online supplemental table 3).

Regarding the extent of denervation, we found a significant correlation with the denervation degree in the lumbosacral

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**Table 2** Clinical features of patients with ALS

<table>
<thead>
<tr>
<th>Patients with ALS (n=148)</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of onset</strong></td>
<td><strong>(N %)</strong></td>
</tr>
<tr>
<td>Bulbar</td>
<td>33 (22.3) ALSFRS-R scale (n=144) 42 (38.2–44.0)</td>
</tr>
<tr>
<td>Spinal</td>
<td>96 (64.9) MRC score (n=147) 4.6 (4.2–4.8)</td>
</tr>
<tr>
<td>Pseudopolyneuritic</td>
<td>11 (7.4) FVC* (n=134) 92.5 (76.7–106.3)</td>
</tr>
<tr>
<td>Pyramidal</td>
<td>8 (5.4) BMI (n=141) 24.6 (22–27.55)</td>
</tr>
</tbody>
</table>

**Clinical phenotype**

| Creatinine (n=139) | 0.78 (0.69–1.05) |

<table>
<thead>
<tr>
<th><strong>Genetic status</strong> (n=142)</th>
<th><strong>(N %)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>C9ORF72 RE carriers</td>
<td>15 (10.6)</td>
</tr>
<tr>
<td>SOD1 mutation carriers</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td>TARDBP mutation carriers</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Definite ALS</td>
<td>24 (16.2)</td>
</tr>
<tr>
<td>Probable ALS</td>
<td>54 (36.5)</td>
</tr>
<tr>
<td>Probable laboratory-supported ALS</td>
<td>33 (22.3)</td>
</tr>
<tr>
<td>Possible ALS</td>
<td>28 (18.9)</td>
</tr>
<tr>
<td>Unclassified (PMA)</td>
<td>9 (6.1)</td>
</tr>
<tr>
<td>Patients with FTD</td>
<td>17 (11.5)</td>
</tr>
</tbody>
</table>

* FVC is expressed as a percentage of the predicted volume. ALS, amyotrophic lateral sclerosis; ALSFRS-R, Revised Amyotrophic Lateral Sclerosis Functional Rating; BMI, body mass index; DD, disease duration; FT9, fine-hand staging; FTD, frontotemporal dementia; FVC, forced vital capacity; m, months; MiToS, Milan-Orino staging; MRC, Medical Research Council; PLMN, predominant lower motor neuron; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy; PUMN, predominant upper motor neuron; RE, repeat expansion; y, years.

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**Figure 1** Distribution of plasma p-tau181 values across the different diagnostic categories. Plasma p-tau181 values in the different diagnostic categories included. Thick lines represent median and IQR. p-tau, phosphorylated tau 181.
Prognostic value of plasma p-tau181 in patients with ALS

Based on univariate Cox regression analysis (134 patients with ALS; 67 dead), ALSFRS-R (p<0.0001), DPR (p<0.0001), FTD status (p=0.042), King’s score (p<0.0001), FVC (p<0.0001), bulbar onset (p=0.004) and plasma p-tau181 (p=0.027) were identified as predictors of the mortality in patients with ALS (online supplemental table 4). Multivariate Cox regression confirmed the value of plasma p-tau181 with baseline plasma p-tau181 levels showed shorter survival (highest tertile of plasma p-tau181 vs lowest tertile: HR 1.90, 95% CI 1.25 to 2.90, p=0.003) as independent predictors of mortality in ALS (figure 2, table 5). Accordingly ALS patients with higher baseline plasma p-tau181 levels showed shorter survival (highest tertile of plasma p-tau181 vs lowest tertile: HR 3.57, 95% CI 1.51-8.41, p=0.004) (online supplemental table 5).

Plasma p-tau181 levels were also confirmed as an independent prognostic factor after including plasma NfL levels in the model (online supplemental table 6).

Longitudinal trajectories of plasma p-tau181 in patients with ALS

No significant differences in the basal plasma p-tau181 values were detected among patients in the three disease progression groups (as calculated by both b- and l-DPR, online supplemental table 5). After stratifying patients with ALS according to the l-DPR, we observed a significant rise in the slopes of p-tau181 values over time (months) in all groups, with the fastest progressing group showing the most consistent increase in the biomarker levels (slow: β=0.025, 95% CI 0.013 to 0.037, p<0.001; intermediate: β=0.014, 95% CI 0.002 to 0.026, p=0.02, fast: β=0.044, 95% CI 0.025 to 0.063, p<0.001, figure 3). A similar rising trend in the biomarker values in all groups was also noted when stratifying patients according to the b-DPR (slow: β=0.021, 95% CI 0.011 to 0.030, p<0.001; intermediate: β=0.026, 95% CI 0.006 to 0.047, p<0.01; fast: β=0.033, 95% CI 0.006 to 0.060, p<0.01).

DISCUSSION

In this study, we confirmed in a large cohort that patients with ALS show significantly elevated plasma p-tau181 levels that in most cases is unrelated to AD pathology. The biomarker change is likely also unrelated to the overall neuroaxonal damage, given the lack of association between plasma p-tau181 and both CSF and plasma NfL. Moreover, the lack of correlation between CSF and plasma p-tau181 strongly suggests a peripheral origin of the biomarker elevation. Our finding of an association between plasma p-tau181 levels and LMN dysfunction supports this interpretation. In contrast to Cousins et al.,25 we quantified the number of regions affected by clinical and/or EMG LMN signs rather than determining the presence or absence of LMN involvement in each region. Using both clinical and EMG assessments rather than the sole clinical evaluation, we could detect a subclinical LMN pathophysiological involvement,29 adding strength to our results. Moreover, we showed that the association remained significant after covarying for potentially confounding clinical factors. Finally, we tested, for the first time, the association of plasma p-tau181 and quantitative EMG correlates of denervation. We found a moderate correlation between plasma p-tau181 levels and the denervation score in the lumbar sacral region but...
Neurodegeneration not in the bulbar and cervical areas. Given the potential peripheral axonal derivation of plasma p-tau181, we speculate that the higher length of the nerve fibres arising from the lumbosacral region, compared with those of bulbar and cervical areas, implying a wider exchange surface with the vascular bed, might explain these results. Another explanation could be that plasma p-tau181 levels reflect the amount of denervated muscular fibres. We also found that patients with a spinal onset and with PLMN or classic phenotypes had significantly higher p-tau181 levels than those presenting with a bulbar onset and a bulbar phenotype, respectively. Notably, unlike Cousins et al,25 we used a standardised phenotype classification based on the clinical longitudinal assessment of patients by expert neurologists besides the UMN-onset or LMN-onset anamnestic distinction.

We also confirmed that patients with ALS with concomitant FTD display lower levels of plasma p-tau181 compared with those with pure motor ALS, making the sporadic reports on a limited tauopathy in ALS-FTD patients17 40 unrelated to plasma p-tau181 concentrations and further indicating a peripheral contribution.

Additionally, in our cohort, plasma p-tau181 levels were elevated in SOD1 mutated patients compared with C9ORF72 and TARDBP patients. With the necessary caution related to the low sample size, these data, in line with those previously reported,25 also support a peripheral contribution to plasma p-tau181 levels, given that the SOD1 ALS phenotype is classically associated with a prevalent LMN degeneration.32

Given the association of plasma p-tau181 with LMN dysfunction, we measured the biomarker in patients with SMA. Our finding of significantly higher plasma p-tau181 values in SMA patients than in controls, with no significant difference with the ALS group, supports the association between LMN involvement and increased plasma p-tau181 levels. Considering the relatively small number of SMA patients, all classified as adult SMA2 and SMA3 patients, a more extensive study, including all SMA types, should confirm these results. Further studies are also needed to investigate plasma p-tau181 levels in other diseases affecting LMN, such as motor axonal neuropathies.

These findings have significant implications for current proposed biomarker strategies to detect early AD pathology in the general population. Evidence indicates that blood-based biomarkers, especially p-tau181 and other p-tau isoforms, can discriminate patients with AD pathology even at a preclinical or prodromal stage.7 26 However, determining if confounding factors affect the blood levels of the biomarker, and maybe even their clinical utility, is necessary before widespread implementation. Our results combined with those of a previous study25 suggest that tau isoforms, likely of peripheral origin rather than brain derived, might represent a significant confounding factor for these assays. Future studies comparing assays targeting

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Plasma p-tau181</td>
<td>1.90 (1.25 to 2.90)</td>
<td>0.003</td>
</tr>
<tr>
<td>Age at onset disease</td>
<td>1.02 (0.99 to 1.04)</td>
<td>0.08</td>
</tr>
<tr>
<td>Onset type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinal</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Bulbar</td>
<td>2.22 (1.11 to 4.42)</td>
<td>0.024</td>
</tr>
<tr>
<td>Pyramidal</td>
<td>0.33 (0.92 to 1.19)</td>
<td>0.09</td>
</tr>
<tr>
<td>Pseudopolyneuritic</td>
<td>0.35 (0.09 to 1.49)</td>
<td>0.15</td>
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<tr>
<td>ALSFRS-R scale</td>
<td>0.96 (0.92 to 1.001)</td>
<td>0.06</td>
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<tr>
<td>FTD status</td>
<td>1.95 (0.86 to 4.46)</td>
<td>0.11</td>
</tr>
<tr>
<td>King’s score</td>
<td>1.72 (0.99 to 2.97)</td>
<td>0.053</td>
</tr>
<tr>
<td>b-DPR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow progressors</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Intermediate</td>
<td>2.49 (1.35 to 4.61)</td>
<td>0.004</td>
</tr>
<tr>
<td>Fast progressors</td>
<td>5.16 (2.40 to 11.07)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ALS, amyotrophic lateral sclerosis; ALSFRS-R, Revised Amyotrophic Lateral Sclerosis Functional Rating; b-DPR, basal disease progression rate; FTD, frontotemporal dementia; p-tau181, phosphorylated tau 181; Ref, reference.
different p-tau and tau isoforms should validate p-tau assays for their specificity for brain-derived p-tau.

In this study, we also showed that plasma p-tau181 levels predict survival in ALS, regardless of other clinical variables already associated with ALS prognosis. Furthermore, we explored the longitudinal behaviour of this biomarker in a subset of patients with ALS, showing a consistent increase in its levels in the disease course, especially in patients with a faster disease progression, as stratified at both basal visits and during the disease course. This is divergent from the longitudinal behaviour of blood NfL, which is stable during the disease course.11 13 15 The longitudinal behaviour of p-tau181 in patients with ALS could reflect the ongoing denervation until the final phase of the disease, with an addictive effect of damage of the peripheral fibres, initially insulted but still undergoing axonal rearrangement.41

CSF and blood NfL probably have a more robust predictive value on survival10–18 than plasma p-tau181 in patients with ALS. Similarly, plasma NfL might be a more promising treatment-monitoring candidate for its stability during the disease course. Nevertheless, the discovery of other reliable biomarkers is valuable due to the recent advances in ALS clinical trials and the highly variable pharmacodynamic targets implied in ALS research.42

Including a significant number of well-characterised patients with ALS with available quantitative electromyographic data is the major strength of our study. The association with survival data and the availability of longitudinally repeatedly sampled patients with ALS constitute a significant added value to our work.

Our study is not free of limitations. First, we could not exclude a concomitant AD pathology neuropathologically, the current gold-standard approach. However, CSF analyses with automated platforms, including the determination of the Aβ42/ Aβ40 ratio, have demonstrated high accuracy in predicting AD pathology in vivo. A second limitation is the lack of standardised time points for the longitudinal sampling of patients with ALS. Finally, the limited number of SMA patients included did not allow us to draw a definitive conclusion on the significance of the trend of increased plasma p-tau181 in these patients.

In conclusion, our study provides evidence that plasma p-tau181 is elevated in patients with ALS and is related to LMN dysfunction, especially at the lumbosacral level. Moreover, plasma p-tau181 levels, likely from a peripheral source, increase progressively in the disease course and predict survival in patients with ALS. Finally, the study further demonstrates that plasma p-tau181 is a less specific AD biomarker than CSF p-tau, making the peripheral source of p-tau a possible confounding factor in the use of this marker for the screening of the general population with cognitive decline.

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