

# Cerebrospinal fluid phosphorylated neurofilament heavy chain and chitotriosidase in primary lateral sclerosis

## INTRODUCTION

Primary lateral sclerosis (PLS) is a rare degenerative disease of upper motor neurons (UMNs) manifesting with progressive spasticity. Disease progression is definitely slower than in amyotrophic lateral sclerosis (ALS). Clinical distinction between PLS and ALS can be challenging, as the UMN-dominant form of ALS may sometimes not manifest lower motor neuron (LMN) signs for a long time after symptom onset.<sup>1</sup> Therefore, traditional PLS diagnostic criteria allowed diagnosis only after 3 or 4 years of documented absence of LMN signs,<sup>2</sup> which poses a psychological burden on patients, hinders correct clinical management and prevents enrolment in clinical trials. In order to overcome these issues, new diagnostic criteria have been recently formulated, shortening the time required for a diagnosis of probable PLS to 2 years.<sup>1</sup>

Diagnostic uncertainties in PLS are complicated by the lack of specific neurochemical biomarkers. Whereas cerebrospinal fluid (CSF) neurofilament levels are clearly increased in ALS, making them a well-established ALS biomarker reflecting axonal degeneration, few studies reported lower increases in PLS<sup>3</sup>; a similar pattern was observed for the putative ALS microglial biomarker chitotriosidase (Chit1).<sup>4</sup> Here we measured phosphorylated neurofilament heavy chain (pNFH) and Chit1 in the CSF of patients with PLS, ALS and non-neurodegenerative neurological conditions, focusing on the ability of each biomarker to distinguish PLS from controls and from ALS.

## PATIENTS AND METHODS

### Patients

In this retrospective study we included those 10 patients (5 men, 5 women) from our consecutive PLS series (n=52) whose CSF was stored in our biobank. They all fulfilled—at the time of sampling or on later evaluations—the recently published diagnostic criteria for definite (n=9) or probable (n=1) PLS.<sup>1</sup> Patients with ALS and neurological controls (NCs) were selected randomly from our biobank to form two cohorts with sex and age distributions similar to PLS. Patients with ALS (n=28; 16 men, 12 women) fulfilled the

revised El Escorial diagnostic criteria of definite or probable ALS.<sup>5</sup> NCs (n=30; 13 men, 17 women) had neurological complaints but no evidence of neurodegenerative diseases (online supplemental table 1).

### Laboratory markers

Centrifuged CSF samples were stored at  $-80^{\circ}\text{C}$  within 2 hours after lumbar puncture. pNFH was measured in all patients; Chit1 was measured in 10 of 10 patients with PLS, 20 of 28 patients with ALS, and 21 of 30 NCs. Commercial ELISAs from Biovendor and MBL, respectively, were used.

### Statistical analysis

Comparisons of continuous variables among >2 groups were performed with Kruskal-Wallis test followed by Dunn's multiple comparisons test, whereas Mann-Whitney U test was used for comparisons between two groups. Diagnostic performances of each single biomarker and of combined biomarkers (sum of z-scores of log-transformed values of both biomarkers) were evaluated with receiver operating characteristic (ROC) curves, choosing cut-offs maximising the Youden index. Correlations between continuous variables were analysed with non-parametric Spearman correlation. The distributions of non-continuous variables in different categories were analysed with the  $\chi^2$  test. Analysis of survival was performed with Kaplan-Meier curves and log-rank test; the endpoint was death or beginning of invasive ventilation for patients who underwent it. The level of statistical significance for all tests was set at  $p < 0.05$ .

## RESULTS

### pNFH and Chit1 in the different diagnostic categories

Patient characteristics are summarised in online supplemental table 2. Median levels of both pNFH and Chit1 differed in the three diagnostic categories, with lowest values in NCs and with patients with ALS showing higher values than patients with PLS (figure 1A,B, online supplemental table 3; individual data in online supplemental table 4). For both biomarkers, the Dunn's pairwise comparisons showed significant differences both between ALS and NCs and between PLS and NCs, but not between PLS and ALS. pNFH and Chit1 correlated with each other in patients with ALS and motor neuron disease (MND) (ie, ALS+PLS)

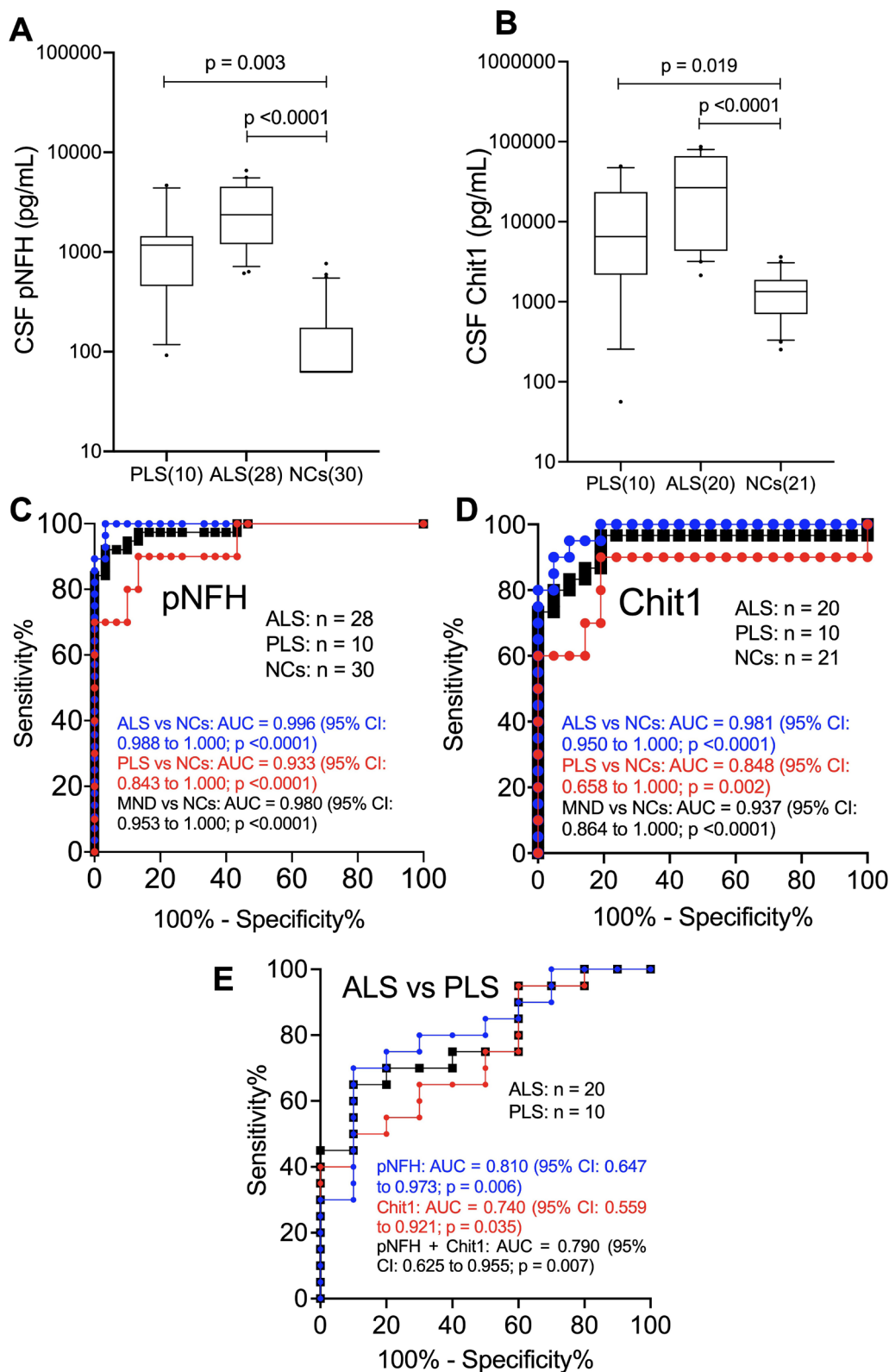
( $r=0.454$  and  $r=0.581$ , respectively). Neither pNFH nor Chit1 differed between patients with PLS, ALS and MND with spinal versus bulbar onset. pNFH correlated positively with age at sampling only in NCs ( $r=0.418$ ) and with progression rate at sampling in patients with ALS ( $r=0.529$ ) and in patients with MND ( $r=0.620$ ), whereas it correlated negatively with disease duration at sampling only in patients with MND ( $r=-0.538$ ). Chit1 showed a positive correlation with age at sampling only in NCs ( $r=0.452$ ) and with progression rate at sampling only in patients with MND ( $r=0.504$ ). Neither pNFH nor Chit1 correlated with Penn UMN Score in patients with PLS, ALS or MND. Finally, in patients with ALS, pNFH levels above the median were negatively associated with survival ( $\text{HR}=4.42$ ), which was not observed for Chit1 (online supplementary figure, online supplemental table 5).

### Diagnostic performance of pNFH and Chit1

Both pNFH and Chit1 demonstrated a good diagnostic performance in distinguishing patients with ALS, PLS and MND from NCs (area under the curve (AUC) for pNFH: 0.996, 0.933 and 0.980, respectively; AUC for Chit1: 0.981, 0.848 and 0.937, respectively) (figure 1C,D). For the differentiation between PLS and ALS, pNFH had an AUC of 0.771, with the best cut-off providing 67.9% sensitivity and 90.0% specificity. The corresponding AUCs for Chit1 and for the combined biomarkers pNFH+Chit1 were 0.740 and 0.790, respectively (figure 1E, online supplemental table 6). Comparison of the ROC curves of pNFH and Chit1 did not show superiority of either biomarker in any diagnostic discrimination, nor did the combined biomarkers outperform either biomarker alone for the differentiation between ALS and PLS (online supplemental table 7).

## DISCUSSION


The main finding of the study is that both pNFH and Chit1 differ in the three diagnostic categories, with PLS showing somewhat intermediate levels between ALS and NCs. Pertaining to pNFH, this could reflect slower degeneration of corticospinal axons in PLS compared with ALS, which is supported by correlation of pNFH with disease progression rate in MND. Similarly, the lower elevation of Chit1 in PLS could reflect a lesser extent of microglial neuroinflammation, which



**Figure 1** CSF pNFH and Chit1 levels in the different diagnostic categories and their discriminatory potential. (A) CSF pNFH levels in patients with PLS, patients with ALS and NCs. (B) CSF Chit1 levels in patients with PLS, patients with ALS and NCs. (C) ROC curves of pNFH for discrimination of ALS versus NCs, PLS versus NCs, and MND (ALS+PLS) versus NCs. (D) ROC curves of Chit1 for discrimination of ALS versus NCs, PLS versus NCs, and MND (ALS+PLS) versus NCs. (E) ROC curves of pNFH, Chit1 and the combined biomarkers pNFH+Chit1 (obtained by summing log-transformed z-scores of both biomarkers) for discrimination of ALS versus PLS. Note that in this case the AUC for pNFH is slightly different from that reported in the text when examining the discriminatory performance of pNFH per se, because in this case we considered only those 20 of 28 patients with ALS having measurements of both pNFH and Chit1 in order to more properly compare the two biomarkers. ALS, amyotrophic lateral sclerosis; AUC, area under the ROC curve; Chit1, chitotriosidase; CSF, cerebrospinal fluid; MND, motor neuron disease; NCs, neurological controls; PLS, primary lateral sclerosis; pNFH, phosphorylated neurofilament heavy chain; ROC, receiver operating characteristic.

in turn may be linked to axonal loss, as suggested by the correlation between the two biomarkers. From a clinical standpoint, in addition to confirming the already known clearly raised CSF levels of pNFH and Chit1 in ALS and the prognostic value of pNFH in this disease,<sup>3</sup> our investigation suggests the potential usefulness of both pNFH and Chit1 for the diagnosis of PLS, although given the limited cohort size it should be regarded as exploratory. Even though pNFH showed higher AUCs compared with Chit1, the discriminatory performances of the two biomarkers were not significantly different: this warrants verification in larger cohorts, which could also enable alternative methods of combination of the two biomarkers. Most importantly, effectiveness of pNFH (or Chit1) in discriminating between PLS and ALS would represent a considerable advance towards early diagnosis of PLS: the latter cannot still be fully achieved even after the introduction of the newer diagnostic criteria, which also await clinical and neuropathological validation.<sup>1</sup> Early diagnosis would expedite enrolment of patients in therapeutic trials designed for PLS, whose paucity is one of the main reasons for the current lack of a specific disease-modifying therapy. Notably, while our ALS cohort was a composite population representing different ALS phenotypes, future studies should focus on comparison between PLS and slowly progressive UMN-dominant forms of ALS. Furthermore, the potential role of pNFH and Chit1 in the diagnosis of PLS suggested by our study deserves further investigation in larger cohorts undergoing prospective (including early) and multimodal (clinical, neurochemical, neuroimaging and neurophysiological) evaluations.

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