Fondazione I.R.C.C.S.
Istituto Neurologico Carlo Besta

The Diagnostic Value of Neurofilament Light in ALS and Its Mimics: a combined CSF and serum study

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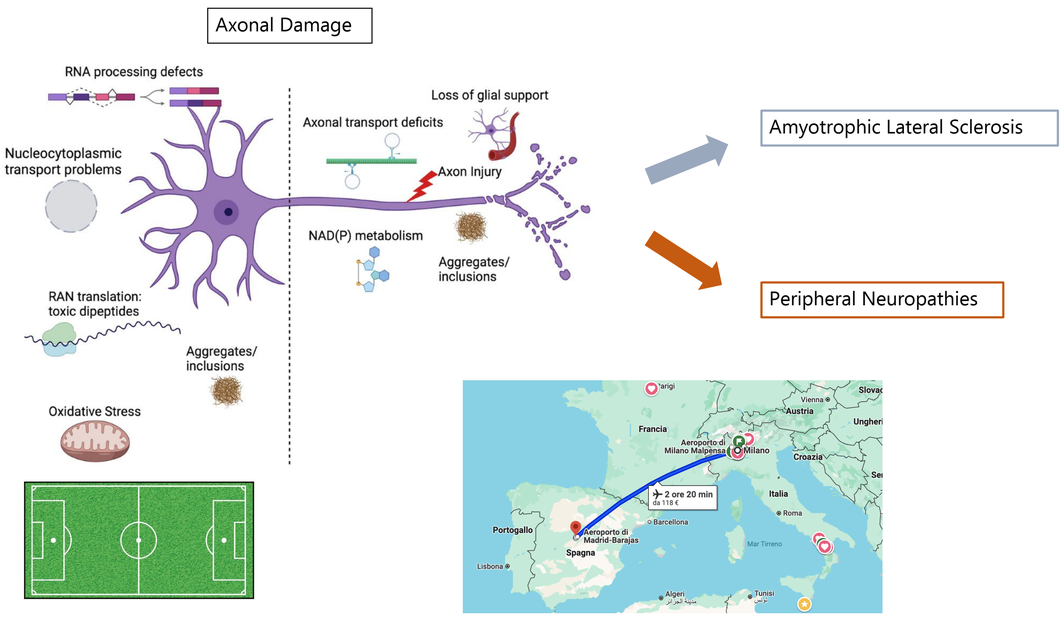
Background:

Neurofilament light chain (NfL) is a well-established biomarker of axonal damage and has shown diagnostic and prognostic potential in amyotrophic lateral sclerosis (ALS). While increased NfL levels are consistently observed in ALS, the distinction between ALS and neuromuscular disease mimics remains challenging, particularly in patients presenting with an atypical phenotype



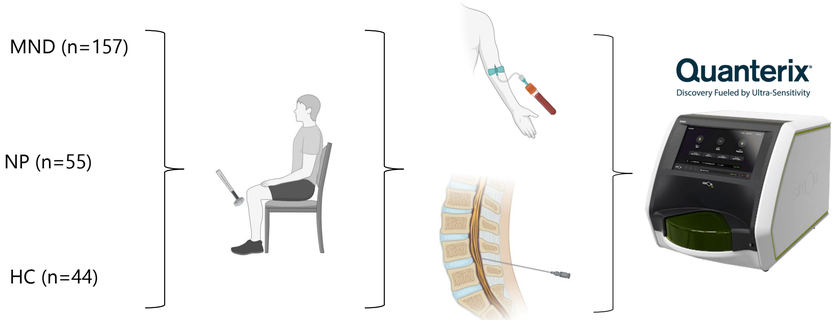
Objective:

The aim of this study was to evaluate both serum and cerebrospinal fluid (CSF) NfL levels in ALS patients compared to those with disease mimics. We also investigated the association between NfL concentrations and disease aggressiveness, survival stratification, and ALS motor phenotypes.

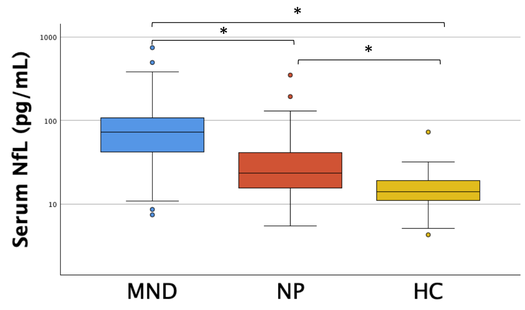
Methods:

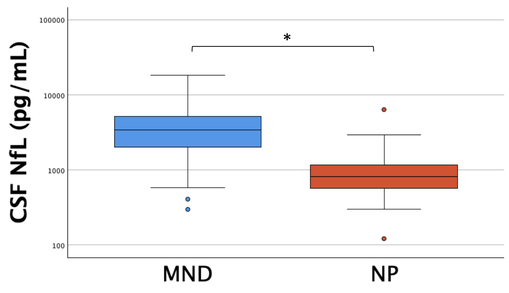
We conducted a cross-sectional study including patients diagnosed with ALS and a control group of patients with neuromuscular disorders. Serum and CSF NfL concentrations were measured using single molecule array (Simoa) technology. Clinical data were collected, including ALS Functional Rating Scale-Revised (ALSFRS-R) scores and disease progression rate, calculated as ΔALSFRS-R. ALS motor phenotypes were classified as classic, bulbar, flail arm, flail leg, pure upper of pure lower motor neuron involvement.

Study population:

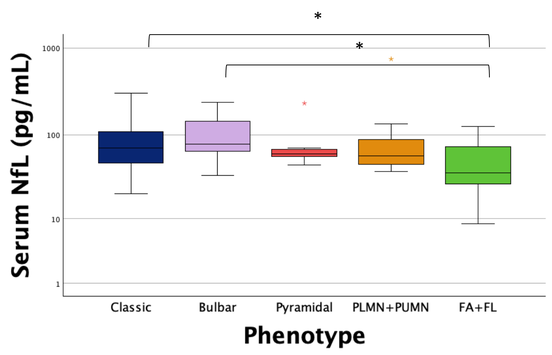


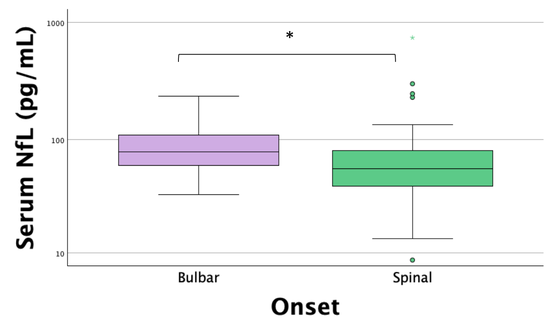
	MND (n=157)	NP (n=55)	HC (n=44)	p
Males/Females	103/54	31/24	24/20	0.289
Age at Diagnosis (years)	61.94 [56.1 - 69.3]	56.9 [51.5 - 69.2]		0.010
Age at Venipuncture (years)	62.1 [56.2 - 69.6]	62,5 [52.9 – 75.1]	63.8 [59.1-70.3]	0.699
Disease Duration (months)	28.3 [10.5 - 32.4]	78.0 [26.3 – 116.5]	\	<0.001
Diagnostic Delay (months)	21.29 [7.1 - 24.0]	37.1 [6.0 – 49.5]	\	0.020
MRC	134.27 [128 – 149]	139.33 [134– 150]	\	0.037
ALSFRS-R	39.49 [36.0 - 44.0]	\	\	
Delta ALSFRS-R	0.6 [0.2 - 0.7]	\	\	
PUMNS	11 [4.0 - 18.0]	\	\	
SMSS	\	46.73 [44.0 – 54.0]	\	
ONLS	\	2.93 [2.0 – 3.0]	\	
ECAS	90.65 [74.25-106]			

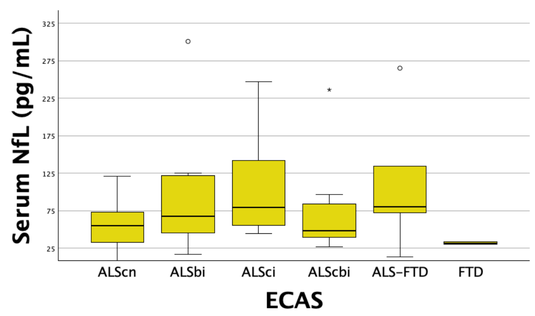




biomarker (pg/ml)	MND (n=150 / 73)	NP (n=51 / 29)	HC (n=44)	ANOVA
Serum NfL	96.28 (94.29) [41.87 - 106.58]	42.22 (56.28) [15.55 - 41.03]	15.81 (10.32) [11.06 - 19.11]	<0.001
CSF NfL	4402.7 (3600.0) [1964.9-5257.2]	1173.9 (1209.2) [2022.7-5173.1]		<0.001



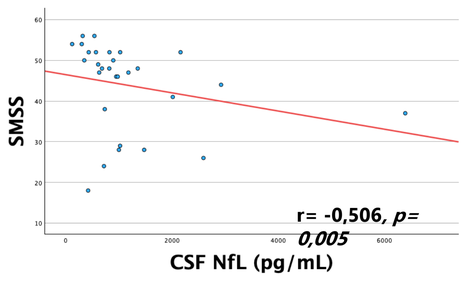


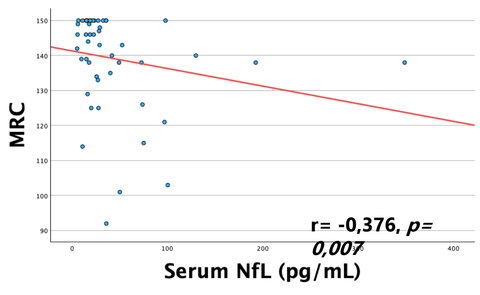


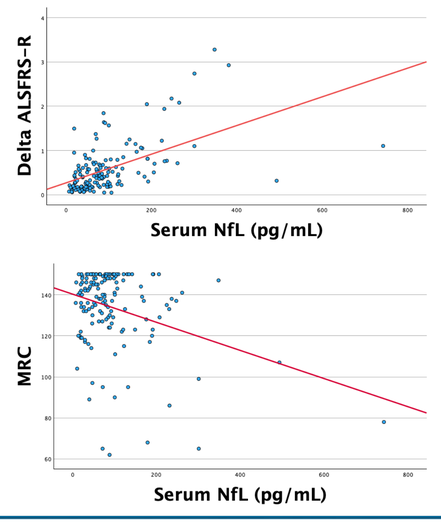
Serum	Bulbar Onset	Spinal Onset	p
NfL	118.4 (68.3)	88.9 (101.5)	<0.001

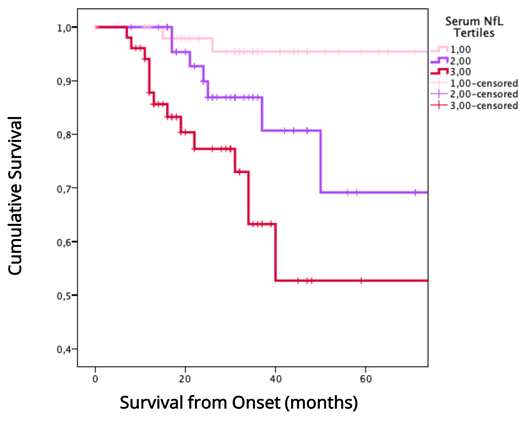
Serum	Classic (37%)	Bulbar (25%)	Pyram. (8%)	PL+PU (15%)	FA+FL (15%)
NfL	116,52 (97,16)	122,55 (73,88)	102,21 (69,80)	87,67 (153,36)	50,22 (39,15)

Clinical correlations in Peripheral Neuropathies









Results:

Both serum and CSF NfL levels were significantly higher in ALS patients compared to those with disease mimics ($p < 0.001$). Within the ALS cohort, higher NfL levels correlated with increased disease aggressiveness, as indicated by a higher ΔALSFRS-R ($r = 0,54$, $p < 0.001$). Furthermore, elevated NfL concentrations stratified patient survival, with higher levels associated with poorer prognosis. HfL serum levels showed a similar performance compared with CSF levels, highlighting the limited added value of CSF NfL measurement.

When analysing motor phenotypes, patients with classic and bulbar-onset ALS exhibited significantly higher NfL levels compared to those with a restricted phenotype ($p < 0.01$). Notably, serum and CSF NfL levels in restricted phenotype did not differ significantly from those measured in neuromuscular disease mimics, underscoring the diagnostic limitations of NfL in these ALS subtypes.

Conclusions:

Serum and CSF mean NfL concentrations are increased in ALS compared to neuromuscular disease mimics and are associated with disease progression and survival. However, in ALS patients with a restricted phenotype, NfL levels present a limited diagnostic yield, highlighting the need for more specific biomarkers to improve diagnostic accuracy in these more challenging presentations

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