Multicenter evaluation of neurofilaments in early symptom onset amyotrophic lateral sclerosis

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Background and research aims

Neurofilaments are elevated in the cerebrospinal fluid (CSF) and serum of ALS patients and have a high relevance in the differential diagnosis (1). In ALS-mutation carriers neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH) are normal before symptom onset and increase at early symptom onset (2). In this study the timing of neurofilament increase in CSF was investigated in patients with sporadic ALS. Therefore CSF NfL and pNfH values were retrospectively related to symptom onset and clinical stage at time of initial diagnosis. To validate diagnosis in the course of disease patients had at least one follow-up investigation.

Conclusions

- CSF and serum NfL and CSF pNfH levels are significantly elevated in ALS patients seen in the first months after symptom onset
- Even in patients who would be clinically characterized as only suspected ALS
- Therefore independent of the clinical diagnostic criteria
- This has been shown across multiple centers

This study further suggests routine measurement of neurofilaments in MND centers. Neurofilaments in ALS strongly enhance the diagnostic accuracy for inclusion into clinical trials in an early disease phase.

Results

Neurofilament levels in early and later symptomatic phase

The concentrations of NfL and pNfH in the CSF and NfL in serum of the early and later symptomatic phase ALS groups were significantly increased compared to all other groups (both p<0.0001; figure 1A, B). CSF NfL and serum NfL concentrations were not significantly different between early and later symptomatic phase, however CSF pNfH concentrations were even higher in early compared to later symptomatic phase ALS patients (p<0.001).

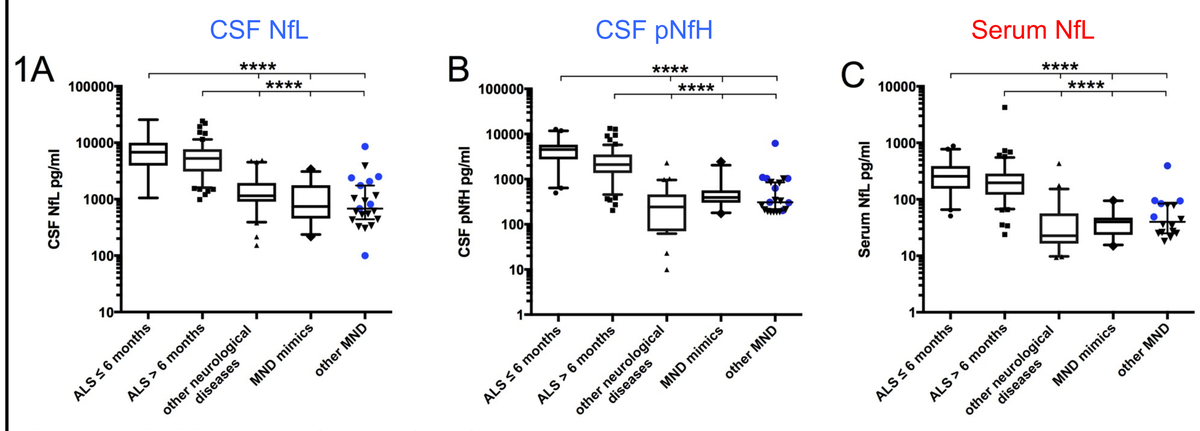

Figure 1A, B, C Early and later symptomatic phase Nf profile
CSF NfL (A), pNfH (B) and serum NfL (C) concentrations. Boxplots show median concentrations, 25% and 75% percentile, 5% and 95% whiskers. Individual symbols represent outliers. Blue symbols mark primary lateral sclerosis cases. ** p<0.01, *** p<0.001, **** p<0.0001

When distinguishing early symptomatic ALS patients from other neurological diseases and MND mimics the area under the curve (AUC) for CSF NfL concentration was 0.95 (0.91-0.99) and 0.94 (0.94-1), sensitivity was 94% (83-99%) and 89% (71-98%) and specificity was 86% (75-93%) and 94% (83-99%) at a cut-off of 2300pg/ml and 2183pg/ml. For CSF pNfH the AUC was 0.99 (0.98-1) and 0.98 (0.95-1), sensitivity was 98% (89-99%) and 78% (58-91%), specificity was 91% (81-97%) and 98% (89-99%) at a cut-off of 625pg/ml and 600pg/ml. For serum NfL the AUC was 0.92 (0.85-0.99) and 0.99 (0.97-1), sensitivity was 88% (73-96%) and 100% (84-100%), specificity was 92% (80-94%) and 90% (76-97%) at a cut-off of 128pg/ml and 97pg/ml).

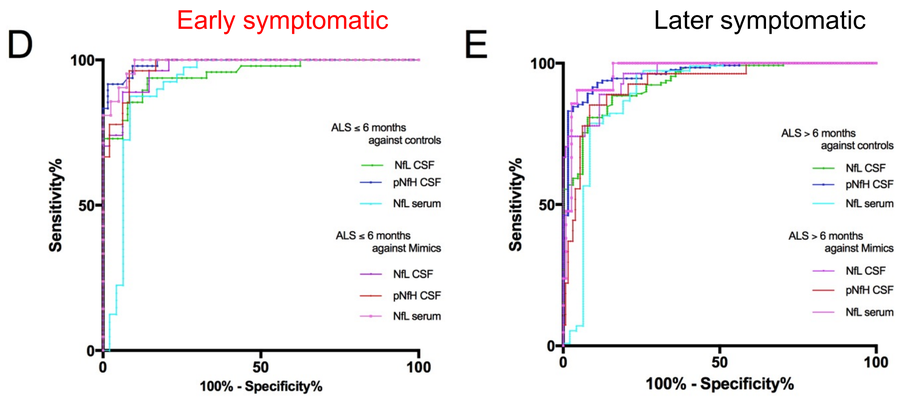

Figure 1D and E Early and later symptomatic phase Nf profile
ROC curves are shown to distinguish early (D) and later (E) ALS from other neurological diseases and MND mimics. ALS, amyotrophic lateral sclerosis; CSF, cerebrospinal fluid; NfL, neurofilament light chain; pNfH, phosphorylated heavy chain.

Neurofilament levels across El Escorial category in early symptomatic ALS patients

There was no significant difference in median CSF NfL and CSF pNfH concentrations between the clinical diagnosis of definite, probable, probable laboratory supported and possible ALS or suspected ALS (p=0.65 and p=0.22). Median serum NfL levels were also not significantly different between El Escorial categories from definite to suspected ALS (p=0.26). From 43 later symptomatic patients who changed category to definite or probable ALS at follow-up median concentrations for CSF NfL, CSF pNfH and serum NfL at baseline did not differ from patients who did not change diagnostic category of ALS at follow-up (p=0.13, p=0.27, p=0.59) (see figure 2B).

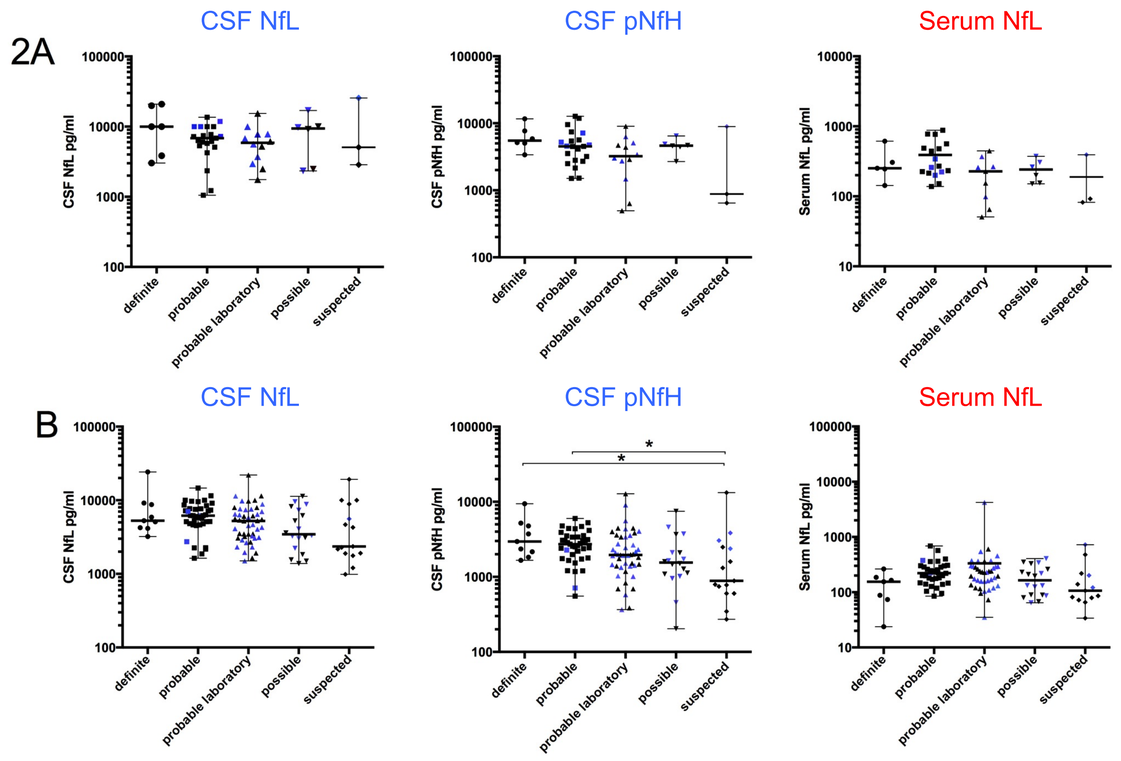

Figure 2 Nf levels according to El Escorial category of ALS
In the early symptomatic phase CSF NfL, pNfH and serum NfL concentrations are not significantly different among the disease categories from definite to suspected ALS (A). In the later symptomatic phase lower CSF pNfH levels are observed in suspected ALS patients (p<0.05) (B). Blue symbols represent change of clinical diagnostic category and black no change of category at follow-up. Data are individual values and bars and whiskers are median and range. *p<0.05.

Neurofilament levels across El Escorial category in later symptomatic ALS patients

In the later symptomatic phase ALS group neither serum nor CSF NfL levels revealed significant differences between clinical ALS phenotypes. However CSF pNfH levels were significantly lower in suspected ALS compared to probable and definite ALS (p<0.05). From 43 later symptomatic patients who changed category to definite or probable ALS at follow-up median concentrations for CSF NfL, CSF pNfH and serum NfL at baseline did not differ from patients who did not change diagnostic category of ALS at follow-up (p=0.13, p=0.27, p=0.59) (see figure 2B).

Materials and methods

Each center had to provide at least 5 CSF and/or serum samples from early symptomatic phase ALS patients with symptom onset ≤6 months from sampling. To minimize center effects 5 CSF and/or serum from later symptomatic phase ALS patients with symptom onset >6 months from sampling, neurological disease controls, patients under differential diagnosis of a MND (mimics) and other MND variants (other MND) were included (for center contributions see table e-1). Patients were seen in a prospective manner in each center. Only patients willing to participate were included. For ALS patients a clinical follow-up visit with a minimum of 3 months was performed. All ALS patients were categorized into clinically definite, probable, laboratory probable, possible according to the revised El Escorial criteria (3) at baseline and follow-up visit. Commercially available ELISA kits were used to measure CSF NfL (IBL, Hamburg, Germany) and CSF pNfH (Biovendor, Heidelberg, Germany).12 For the measurement of serum NfL we used Simoa platform which is the same methodology as the one employed in a previous study. For NfL the detector antibody anti-NfL mAB 2:1 and capture antibody anti-NfL mAB 47:3 were applied. 54 CSF and 45 serum samples were analyzed from patients with early symptomatic phase ALS and 135 CSF and 118 serum samples from later symptomatic phase ALS patients. The control group comprised 65 CSF and 48 serum samples from patients with other neurological diseases, 27 CSF and 21 serum samples from MND mimics and 21 CSF and 16 serum samples from patients with other MND.

References

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