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 NF and pNF 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.

Results

Neurofilament levels in early and later symptomatic phase

The concentrations of NF and pNF 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 pNF concentrations were even higher in early compared to later symptomatic phase ALS patients (p<0.001).

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% (79-91%) and specificity was 86% (75-93%) and 94% (83-99%) at a cut-off of 2300pg/ml and 2183pg/ml. For CSF pNF 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.

Conclusions

- CSF and serum NFL and CSF pNF 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.

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 late 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 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. Commerically available ELISA kits were used to measure CSF NFL (IBL, Hamburg, Germany) and CSF pNF (Biovendor, Heidelberg, Germany). For the measurement of serum NFL we used Simoa platform which is the same methodology as the one employed in a previous study. For pNF, 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 other neurological diseases, 27 CSF and 21 serum samples from MND mimics and 23 CSF and 16 serum samples from patients suffering from familial or sporadic ALS.

References


Acknowledgments: This work was supported by BMFB (Federal Ministry of Education and Research, Germany); Competence net neurodegenerative diseases (project: FTLDs), the MND-net, the NMDP networks for standardization of biomarkers (BiomarkAPD, Tepha); the EU (NADINE), foundation of the state of Baden-Württemberg, BMF (Federal Ministry for Economic Affairs and Energy) and BIZU.

Figure 1A, B, C Early and later symptomatic phase NFL profile

Figure 2A, B, C Early and late symptomatic phase pNF profile

Figure 3A, B, C Early and later symptomatic phase NFL profile

Figure 4A, B, C Early and later symptomatic phase pNF profile