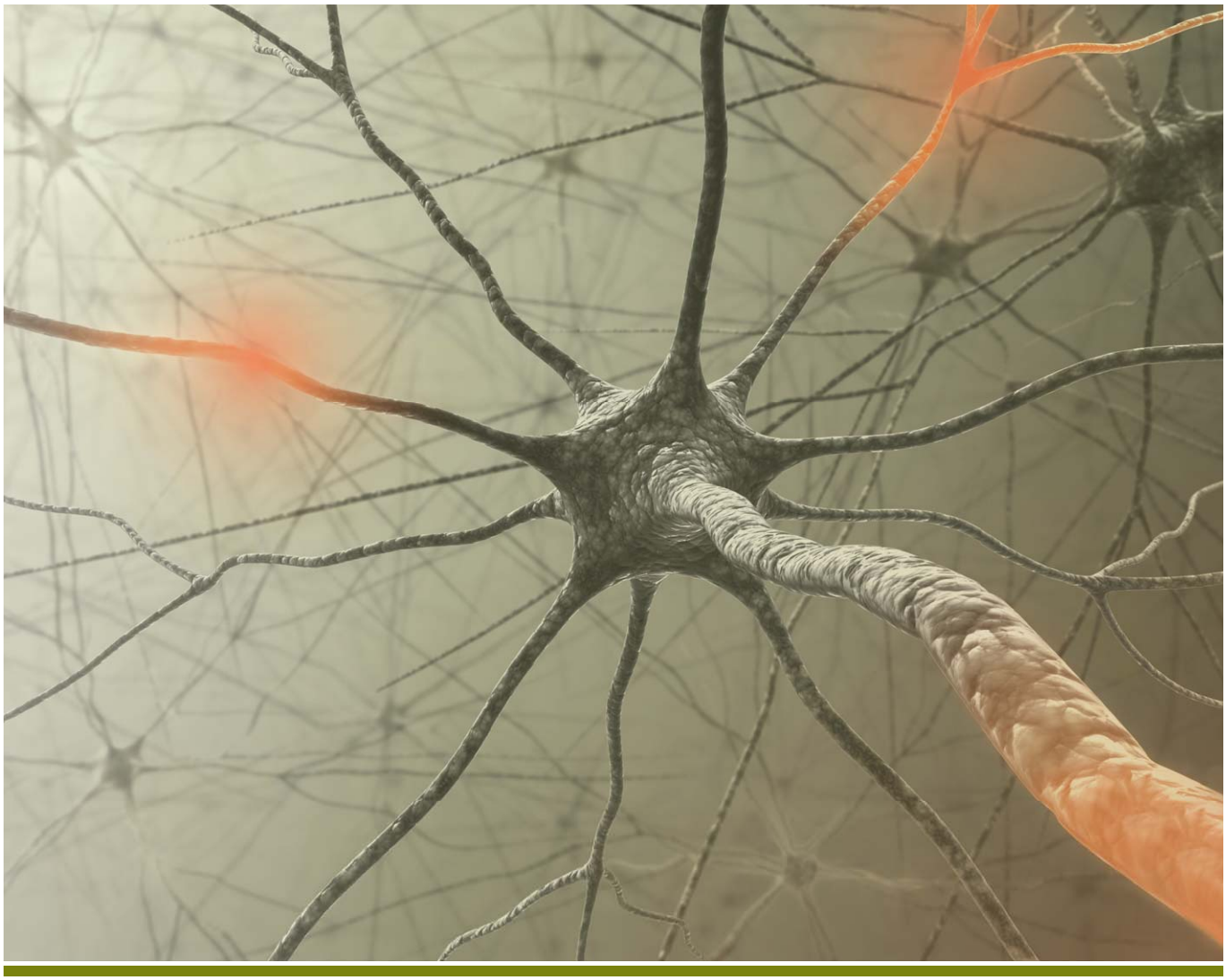
EUROIMMUN Medizinische Labordiagnostika AG

# Neurofilaments in diagnostics

## Biomarkers for neuroaxonal damage



- **Neurofilament concentrations are increased especially in rapidly progressive neurodegenerative diseases**
- **Neurofilaments are potential biomarkers to support early diagnosis and thus treatment of symptoms in amyotrophic lateral sclerosis (ALS)**
- **EUROIMMUN ELISAs allow analysis of serum neurofilament (pNfH) as part of ALS routine diagnostics**

## Neurofilaments

Neurofilaments (Nf) occur exclusively in neurons and frequently in myelinated axons. As part of the cytoskeleton they provide structural stability and allow effective and rapid transmission of stimuli. With a diameter of 10 nm, Nf are intermediary filaments and mainly consist of three subunits: NfL (light), NfM (medium) and NfH (heavy). Due to its specific amino acid sequence at the C terminus, NfH is particularly strongly phosphorylated (pNfH). Alongside other posttranslational modifications, this is important for the composition of Nf (see Fig.1).[1, 2]

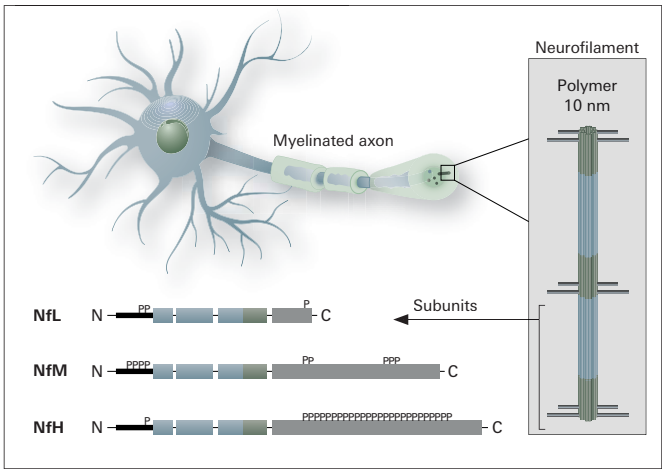



Fig. 1: Structure and composition of neurofilaments (modified from Khalil et al., 2018), P: phosphorylation

## Marker for neuroaxonal damage

In neurological diagnostics, Nf – particularly NfL and pNfH – are attracting increasing attention as biomarkers since they are released in the case of neuroaxonal damage independently of the underlying pathophysiological process and reach abnormal concentrations both in cerebrospinal fluid (CSF) and blood. This may occur, for instance, in inflammatory, neurodegenerative, vascular and traumatic diseases. Studies indicate that NfL and pNfH values increase independently of each other in various diseases (see Fig. 2).[3,4] The concentrations are especially high in rapidly progressive neurodegenerative diseases. For this reason, NfL and pNfH are being thoroughly researched, for instance, as potential biomarkers for amyotrophic lateral sclerosis (ALS).[1, 2]

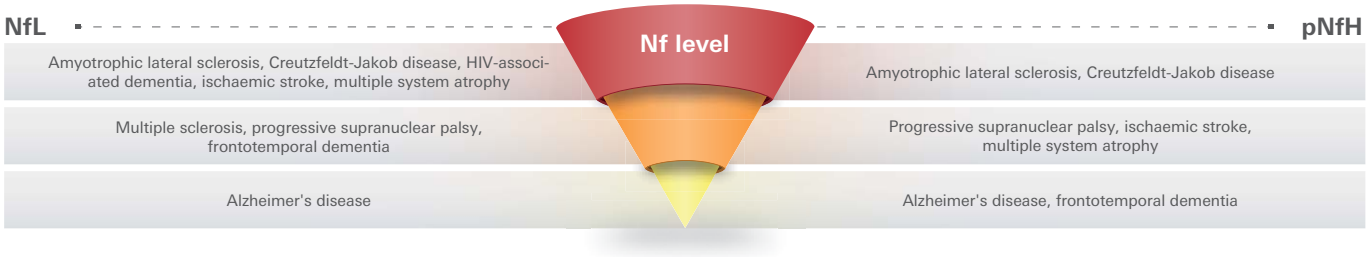



Fig. 2: Comparison of the increase in NfL and pNfH concentrations in selected diseases

## Amyotrophic lateral sclerosis (ALS)

With an incidence of around 2/100,000 per year, ALS is the most frequent motor neuron disease (MND). It is characterised by progressive degeneration of the upper and lower motor neurons, which leads to progressive muscle weakness and finally to a complete loss of autonomy and the ability to communicate. ALS generally occurs between 50 and 60 years of age and cannot be cured. The majority of patients succumb to the disease after three to five years following the onset of symptoms. Due to the continuously ageing population, ALS cases are projected to increase by 69% by 2040. To date, the diagnosis of ALS has been based only on the clinical evaluation of symptoms and electromyography (EMG). Specific biomarkers are therefore urgently needed for early identification of the disease and thus treatment of symptoms as well as for the selection of patients for clinical studies.

## The role of neurofilaments in ALS diagnostics

The potential of Nf in differential diagnostics, for instance in ALS, could be shown in a large prospective study. Interestingly, compared to patients with MND mimics and neurological control groups, ALS patients exhibited increased concentrations only of NfL and pNfH, but not of the two neuronal destruction markers total tau (T-tau) and phospho tau (P-tau). Based on the Nf values, it was possible to differentiate ALS patients from controls with a high positive predictive value.[3] Moreover, a further study revealed that the Nf concentrations were comparatively higher in ALS patients with a medium to rapid (versus slow) disease progression and the more the upper and lower motor neurons were affected.[5]

## Prognostic markers

In a survival time analysis, ALS patients were divided into two groups, depending on the Nf concentration in the CSF at the time of diagnosis. The differences in the survival times between patients with a high and patients with a low Nf level were significant for NfL and pNfH (see Fig. 3) and allow the following interpretation: The higher the Nf level, the poorer the survival prognosis.[6] This tendency has been confirmed by further studies.[2,3]

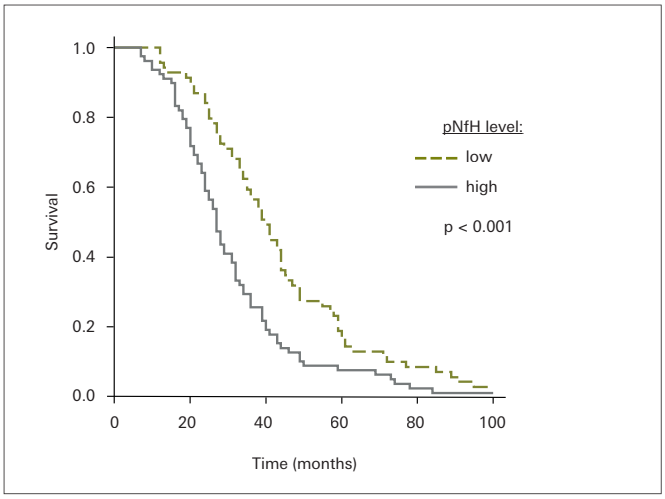



Fig. 3: Survival curves of ALS patients depending on the CFS pNfH concentrations (modified from Rossi et al., 2018)

## pNfH versus NfL

ROC analyses from several recent studies show that CSF pNfH is a more suitable biomarker in ALS than CSF NfL. For CSF pNfH the specificity and sensitivity as well as the positive and negative predictive values (PPV or NPV) were higher for the differentiation both from other neurological diseases and from MND mimics (see Fig. 4).[5-7]

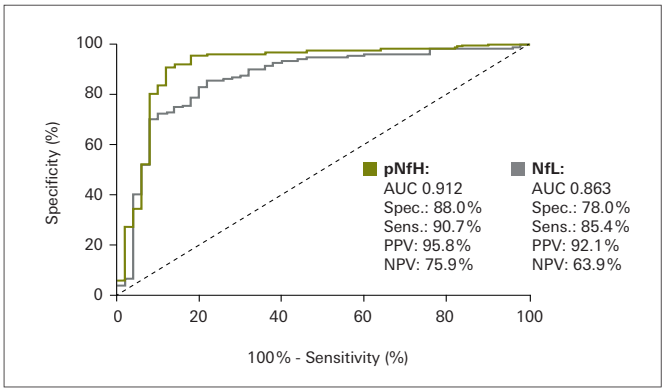



Fig. 4: Differentiation potential of NfL and pNfH in patients with ALS versus MND mimics (modified from Poesen et al., 2017)

## CSF versus blood

The concentration of Nf in blood is around ten times lower than in CSF. In a study the pNfH values were examined in the blood of ALS patients using a highly sensitive ELISA and compared with those obtained with CSF samples. This showed a good correlation of results.[8] The relationship between pNfH concentrations in the blood and serum are not yet fully understood. Since CSF samples from ALS patients in the early disease stage are rarely available, however, the analysis of serum pNfH may provide early information, which could be verified further by subsequent measurement of CSF pNfH.

## Prediagnostic potential of serum pNfH

In a study, the serum concentrations of pNfH in patients with primary sporadic ALS, compared to healthy subjects, were significantly increased up to 18 months prior to diagnosis and kept increasing further until diagnosis (see Fig. 5). The pathophysiological processes in ALS start well before the appearance of clear symptoms.[9] However, diagnosis is only established with a delay of more than one year following first manifestations, as was shown by investigations (see Fig. 5).[10]

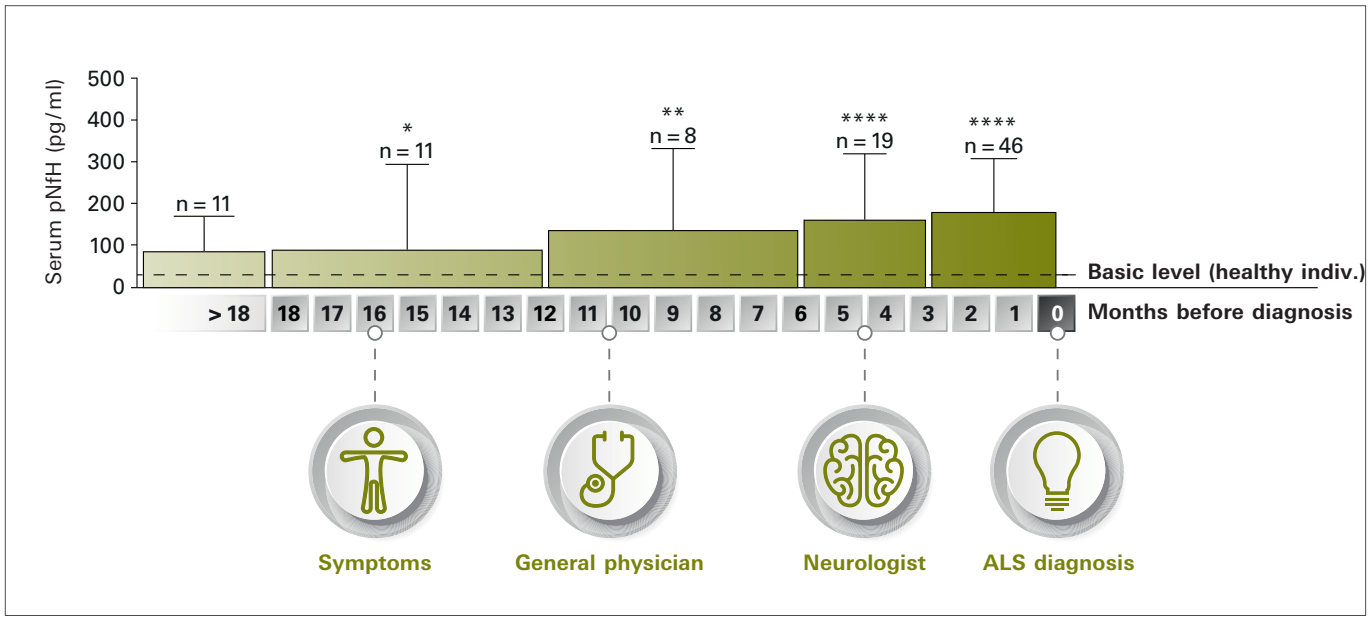



Fig. 5: Comparison of prediagnostic serum pNfH concentrations and diagnostic course (modified from De Schaepdryver et al., 2019 and Galvin et al., 2015).

## Test systems from EUROIMMUN

EUROIMMUN is the only provider of ELISAs for pNfH measurement licensed for in vitro diagnostics. Its two CE-marked tests differ primarily with respect to their sensitivity, which enables the analysis of CSF as well as serum and plasma samples. Moreover, these tests can be performed more quickly, even fully automatically using the EUROIMMUN Analyzer I or I-2P or the EUROLabWorkstation ELISA. This allows the use of pNfH in ALS routine diagnostics to support a precise diagnosis.


### Neurofilament (pNf-H) ELISA

- Optimal for CSF samples
- Very quick analysis in only 2 1/4 hours
- Order no.: EQ 6561-9601

### Neurofilament (pNf-H) ELISA, highly sensitive

- Optimal for serum and plasma samples
- Most sensitive ELISA for pNfH on the market
- Order no.: EQ 6562-9601

## In a nutshell

- Neurofilaments (Nf) occur exclusively in neurons and are released into the CSF and blood in case of neuroaxonal damage.
- Increased Nf concentrations are observed, for instance, in motor neuron diseases (MND) such as amyotrophic lateral sclerosis (ALS).
- A high potential for differentiation of ALS from MND mimics and neurological control groups could be shown particularly for pNfH – both in CSF and serum samples.
- The use of serum pNfH as prediagnostic biomarker in suspected cases of ALS might support early diagnosis and allow quicker treatment of symptoms as well as participation of patients in studies.
- The CE-marked ELISAs from EUROIMMUN help to establish the analysis of pNfH in routine diagnostics.

## References

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