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REPORT

Phosphoneurofilament heavy chain and vascular endothelial growth factor as cerebrospinal fluid biomarkers for ALS

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Introduction

The most promising molecular markers for ALS are neurofilaments, namely phosphorylated neurofilament heavy chain (pNFH). In ALS, increased level of pNFH has been reported in the CSF (1,2), in particular in fast progressors (3), with a negative correlation with disease duration (4).

VEGF has been associated with ALS pathogenesis (5). Decreased levels were described in the CSF and plasma of ALS patients (6), but the results are inconsistent (1).

Here we explore the diagnostic potential of combining pNFH and VEGF as ALS biomarker.

Population and methods

Groups of 36 ALS patients with probable or definite disease (revised El Escorial criteria) and 15 patients with other neurological disorders were studied (Table I). All patients gave written consent; the protocol was approved by the ethics committee.

CSF was collected by lumbar puncture into polypropylene tubes and kept at −80°C (7). pNFH (8) and VEGF (9) were quantified by ELISA (BioVendor Research and Diagnostic Products, Czech Republic, no. RD191138300R and R&D Systems, QuantiGlo, no. QVE00B, respectively).

Statistical analysis was performed using GraphPad Prism 6.

Results

pNFH level was higher in ALS patients than in controls (median 1739 pg/ml vs. 417.4 pg/ml, respectively; \( p = 0.0020 \)) (Table I) (Supplementary Figure 1A). ROC analysis showed an area under the curve (AUC) of 0.7704 (Figure 1). A cut-off value of 554 pg/ml for pNFH generated sensitivity of 75% and specificity of 60% were recorded. pNFH levels correlated negatively with disease duration and positively with rate of functional decline [(40-ALSFRS)/disease duration (months)] (Supplementary Figure 1B, C). Higher pNFH levels were observed in patients with short disease duration (<1 year) (Supplementary Figure 1D).

VEGF levels were lower in patients than in disease controls (median 6.7 pg/ml vs. 10.9 pg/ml, \( p = 0.0356 \)) (Table I) (Supplementary Figure 2). ROC analysis showed AUC 0.6870 (Figure 1).

pNFH/VEGF ratio in ALS patients was higher than in controls (median 224.3 and 54.6, respectively) (\( p < 0.0001 \)) (Supplementary Figure 3A). ROC analysis showed AUC 0.8481 (Figure 1). A cut-off value of 75.02 for pNFH/VEGF ratio increased the sensitivity (83.3%) and the specificity (80.0%). Significant correlations between pNFH/VEGF ratio and disease duration (negative), rate of functional decline or ALSFRS-R (positive) were found (Supplementary Figure 3B–D). Furthermore, ALS patients with short disease duration displayed
Discussion

We have observed that pNFH levels were increased in the CSF and correlated with the rate of disease progression and disease duration, which is in agreement with other reports (1–3). Concerning diagnostic value, we have explored the diagnostic value of combining both biomarkers. We have observed that higher levels of pNFH/VEGF ratio (Supplementary Figure 3E).

Table 1. Demographic and clinical features of the patients included.

<table>
<thead>
<tr>
<th>Gender (M/F)</th>
<th>Age (years)</th>
<th>Disease duration (years)</th>
<th>ALS/FRS</th>
<th>FVC (% predicted value)</th>
<th>Onset site (bulbar/spinal)</th>
<th>Total protein (mg/mL)</th>
<th>pNFH [VEGF] (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>7/8</td>
<td>64.1 (55.3–67.5)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.48 (0.39–0.58)</td>
<td>417.4 (243.5–843.9)* [10.9 (7.7–13.0)]#</td>
</tr>
<tr>
<td>ALS</td>
<td>26/10</td>
<td>56.0 (49.1–63.2)</td>
<td>0.87 (0.58–1.92)</td>
<td>36 (34–38)</td>
<td>104 (90–117)*</td>
<td>4/31</td>
<td>0.48 (0.37–0.58)</td>
</tr>
</tbody>
</table>

Notes:
1. The control group was formed by patients with the final diagnosis of chronic inflammatory demyelinating neuropathy, hereditary demyelinating polyneuropathy, diabetic neuropathy, axonal polyneuropathy, brachial plexitis, myelitis, atypical headache, normal pressure hydrocephalus, undetermined spinal cord lesion, multiple sclerosis and multiple system atrophy.
2. Demographic and clinical information presented as median and between brackets the interquartile range IQR (25% percentile–75% percentile).
3. Testing for normality was performed using the D’Agostino & Pearson omnibus normality test. The non-parametric Mann–Whitney test was applied to determine statistical differences between two groups. p values <0.05 were considered as statistically significant.
4. *p = 0.0020; #p = 0.0356.

Values calculated for a subgroup of 30 patients with measured FVC.
both abnormal in ALS (1,2,6). We found that this index has a potentially higher diagnostic accuracy than the single evaluation of pNFH. Higher CSF pNFH/VEGF ratio was associated with a more aggressive disease course and respiratory failure, probably explained by the combination of rapid motor neurons death and deregulation in the synthesis of VEGF in ALS.

Our results underscore the potential of CSF pNFH as a diagnostic and prognostic biomarker in ALS and combined analysis of pNFH/VEGF could provide higher diagnostic yield. This should be investigated in a larger population of patients.

Supplementary material for this article is available via the supplementary tab on the article’s online page at http://dx.doi.org/10.1080/21678421.2016.1212894.

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Declaration of interest
The authors report no conflicts of interest.

References

Supplementary material available online