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

MARGARIDA GONÇALVES¹, MAMEDE DE CARVALHO²,³, CRISTINA PEIXOTO¹,⁴, PAULA ALVES¹,²,³,⁴, CARMO BARRETO¹,⁴, ABEL OLIVA¹,⁴, SUSANA PINTO³, ANA LABORINHO-PRONTO³, MARTA GROMICHO³ & JÚLIA COSTA¹

¹Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal, ²Hospital de Santa Maria, Centro Hospitalar Lisboa-Norte, Lisbon, Portugal, ³Instituto de Medicina Molecular, Faculty of Medicine, University of Lisbon, Portugal, and ⁴Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

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 $\sim$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...
higher levels of pNFH/VEGF ratio (Supplementary Figure 3E).

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 specificity and sensitivity our values are slightly lower than those reported elsewhere (2), which might be due to the different populations analysed and number of subjects. On the other hand, in agreement with others (6) we observed a lower level of VEGF in ALS CSF.

To our knowledge pNFH and VEGF are not interdependent; however, we have explored the diagnostic value of combining both biomarkers,
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.

Acknowledgements

We wish to thank the participants in this study. This work was supported by EU JPND project SOPHIA, Fundação para a Ciência e a Técnologia (FCT) [JPND/0003/2011], Portugal; Euronanomed 2 ERA-NET project GlioEx, FCT [ENMed/0001/2013]; iNOVA4Health, UI FCT/Ministério da Educação e Ciência [UID/Multi/04462/2013].

Declaration of interest

The authors report no conflicts of interest.

Supplementary material available online

References

1. Costa J, de Carvalho M. Emerging molecular biomarker targets for amyotrophic lateral sclerosis. Clin Chim Acta. 2016;455:7–14.
2. Steinacker P, Feneberg E, Weishaupt J, Bretschneider J, Tumani H, Andersen PM, et al. Neurofilaments in the diagnosis of motor neuron diseases: a prospective study on 455 patients. J Neurol Neurosurg Psychiatry. 2016;87:12–20.
3. Boylan KB, Glass JD, Crook JE, Yang C, Thomas CS, Desaro P, et al. Phosphorylated neurofilament heavy subunit (pNF-H) in peripheral blood and CSF as a potential prognostic biomarker in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2013;84:467–72.
4. Ganesalingam J, An J, Bowser R, Andersen PM, Shaw CE. pNfH is a promising biomarker for ALS. Amyotroph Lateral Scler Frontotemporal Degener. 2013;14:146–9.
5. Oosthuyse B, Moons L, Storkebaum E, Beck H, Nuyens D, Brusselmans K, et al. Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. Nat Genet. 2001;28:131–8.
6. Devos D, Moreau C, Lassalle P, Perez T, De Seze J, Brunaud-Danel V, et al. Low levels of the vascular endothelial growth factor in CSF from early ALS patients. Neurology. 2004;62:2127–9.
7. Teunissen CE, Petzold A, Bennett JL, Berven FS, Brundin L, Comabella M, et al. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology. 2009;73:1914–22.
8. Gonçalves M, Tillack L, de Carvalho M, Pinto S, Conradt HS, Costa J. Phospho-neurofilament heavy chain and N-glycomics from the cerebrospinal fluid in amyotrophic lateral sclerosis. Clin Chim Acta. 2015;438:342–9.
9. Carilho R, de Carvalho M, Swash M, Pinto S, Pinto A, Costa J. Vascular endothelial growth factor and amyotrophic lateral sclerosis: the interplay with exercise and non-invasive ventilation. Muscle Nerve. 2014;49:545–50.