Metabolic and fluid biomarkers support a microglia-mediated inflammatory signature in ALS

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1 Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by motor neuron degeneration and characterised by an incidence of 1 – 2.6 new cases per 100.000 person-years and a survival of 2 – 4 years after the onset of symptoms. The pathogenesis is extremely complex and involves the interaction of intrinsic factors (genetic mutations, altered neuronal and/or glial functionality, neuro-inflammation and excitotoxicity)[1] and extrinsic factors (i.e. environmental contaminants, traumatic events, cigarette smoking). To better understand ALS pathogenesis and clinical evolution, specific biomarkers are needed: both liquid-based markers from blood or cerebrospinal fluid (CSF) and neuroimaging techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET) have shown promise. Particularly, activated microglia might play an essential role in the perpetuation of motor neuron damage, which, as advanced imaging studies show, appears in the form of areas of altered cerebral metabolism. The identification of neuro-inflammation markers and correlated PET-MRI patterns could represent a valuable system for diagnostic support and prognostic definition for ALS [2-3].

2 Aim of the study

Here, we combine clinical measurements, fluid biomarkers, and PET/MRI metabolic analysis to unravel the role of reactive microglia in Amyotrophic Lateral Sclerosis pathogenesis.

3 Materials and Methods

Based on specific inclusion and exclusion criteria, 25 cases and 10 healthy controls were recruited between 2017 and 2021 and underwent cerebrospinal fluid sampling to determine concentrations of glycosyl hydrolases produced by reactive microglia known as chitinases (chitotriosidase 1 – CHT1 – and chitinase-3-like protein 2 – CHI3L2), Tumor Necrosis Factor α (TNF-α), Interleukin 6 (IL-6) and neurofilaments (NFL). ^{18}F-FDG PET-MRI scan was also performed in all patients to explore brain metabolism. The analysis results were integrated with the clinical data (disease phenotype, age at symptoms onset, sex, ALS Functional Rating Scale-revised (ALSFRS-r) and its monthly variation in time (Δ ALSFRS-r), and any known genetic mutation) of the patients.

	ALS Patients	Healthy Controls
Total	25	10
Male / female, n (%)	M 12 (48%) F 13 (52%)	M 2 (20%) F 8 (80%)
Spinal / bulbar phenotype, n (%)	Spinal 21 (84%) / Bulbar 4 (16%)	/
Known genetic mutations, n (%)	ALS2 gene, 2 (8%)	/
Age at symptom onset, mean (SD)	69.39 (9.40)	/
Age at CSF analysis, mean (SD)	61.61 (9.49)	49.80 (12.73)
Age at PET-MRI, mean (SD)	61.61 (9.49)	49.80 (12.73)
ALSFRS-r at T_0, mean (SD)	35.75 (0.71)	/
Δ ALSFRS-r, median (IQR)	-0.02 (-0.03 – -0.01)	/

Table 1. Main demographic and clinical data. M: male. F: female. SD: standard deviation. T0: time of the first evaluation. IQR: interquartile range.

4 Results

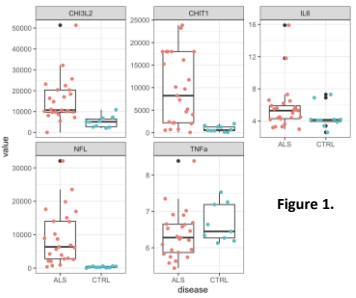

Figure 1.

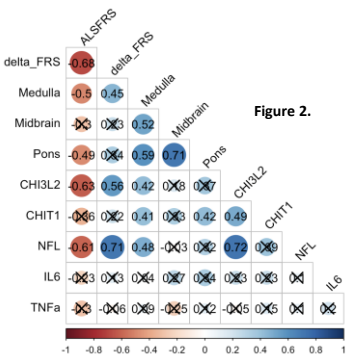

Figure 2.

Figure 1. *CSF levels of neuroinflammatory biomarkers in ALS patients and healthy controls.. Compared to controls, increased CSF levels of NFL, IL-6 and CHITs has been found in ALS patients. CTRL: controls.* * p-value < 0.05.

Figure 2. *Correlation between biofluid and metabolic markers highlight a positive correlation between medulla oblongata metabolism and both CHITs and NFL, as well as a negative correlation between ALSFRS-r and CHI3L2 and NFL.* Not crossed results are statistically significant (p-value < 0.05).

5 Discussion and Conclusions

First, we found that patients present with higher CSF levels of NFL and CHITs compared to controls. Moreover, the levels of CHIT1 correlate with the medulla oblongata and pons metabolism, and CHI3L2 with medulla oblongata metabolism. From a clinical perspective, CHI3L2 correlates both with the ALSFRS-r scores at the first visit and with the Δ ALSFRS-r score. Our findings suggest a potential relationship between microglial activation and disease severity, further strengthening the hypothesis that neuroinflammation and activated microglia play a central role in ALS pathogenesis. These considerations could enhance the foundations for the research and development of specific targeted therapies.

References

1. Thompson AG, Gray E, Bampton A, Raciborska D, Talbot K, Turner MR. CSF chitinase proteins in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2019 Nov;**90**(11):1215-1220.
2. Vu L, An J, Kovalik T, Gendron T, Petrucelli L, Bowser R. Cross-sectional and longitudinal measures of chitinase proteins in amyotrophic lateral sclerosis and expression of CHI3L1 in activated astrocytes. J Neurol Neurosurg Psychiatry. 2020 Apr;**91**(4):350-358.
3. Jamali AM, Kethamreddy M, Burkett BJ, Port JD, Pandey MK. PET and SPECT Imaging of ALS: An Educational Review. Mol Imaging. 2023 Aug **19**;2023:5864391.