Circulating Cytokines and Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that has no effective treatment. The lack of any specific biomarker that can help in the diagnosis or prognosis of ALS has made the identification of biomarkers an urgent challenge. Multiple panels have shown alterations in levels of numerous cytokines in ALS, supporting the contribution of neuroinflammation to the progressive motor neuron loss.

cytokines biomarkers amyotrophic lateral sclerosis

1. Introduction

Amyotrophic lateral sclerosis (ALS) is one of the most serious motor neuron diseases, and the most common adult motor neuron disease. It is characterized by loss of the cortex, brainstem, and spinal cord motor neurons (MNs), leading to muscle paralysis, and finally premature death due to respiratory failure within 2–5 years after diagnosis. Unfortunately, to date, no effective therapies able to cure the disease are available. More than 90% of ALS cases are sporadic with unknown causes. On the other hand, around 10% of ALS patients have a family history, involving mutations in a number of genes, such as Cu^{2+}/Zn^{2+} superoxide dismutase 1 (SOD1), TAR DNA binding protein 43 (TDP-43), fused in sarcoma (FUS) and chromosome 9 open reading frame 72 (C9ORF72) repeat expansions [1]. These mutations found in ALS have allowed the development of animal models that are helpful in the study of this disease. One of the best characterized animal models for ALS is the SOD1G93A mouse model, which carries a G93A mutation (substitution of Glycine to Alanine at codon 93) in the human SOD1 gene, and presents both clinical and pathological characteristics of ALS patients [2].

2. The Necessity of Identifying Biomarkers for ALS

There is an imperious need to identify biomarkers that can help diagnose the disease at earlier stages and also to predict the course of the disease that allow following of more accurate therapeutic strategies. The ideal biomarker should be sensitive enough to diagnose ALS at the pre-symptomatic stage; specific for ALS and able to discriminate from other clinically similar neurodegenerative diseases; able to predict the progression of the disease in each patient; and easily accessible and applicable for all patients, despite their physical condition [3]. Although the origin of ALS remains unknown, multiple panels of biomarkers have been described in ALS patients and murine models to explain the progressive motor neuron loss and muscle atrophy, including imaging, electrophysiological and wet biomarkers [4].

Neuroimaging biomarkers allow a faithful visualization of the structural alterations happening in the tissue of study when comparing them with healthy tissues. Different brain imaging techniques have been used to detect these pathological changes in ALS patients, such as magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), diffusion tensor imaging (DTI) and positron emission tomography (PET) [5][6]. As a result of using these techniques, some pathological alterations regarding cortical atrophy, neuronal integrity and brain white matter abnormalities have been positively correlated with progression of the disease or resulted helpful in the diagnosis [7][8][9][10]. Although some promising imaging biomarkers have been found, none of them is fully sensitive and specific enough for ALS diagnosis or prognosis.

Numerous neurophysiologic biomarkers have been identified and proposed as biomarkers that help in the early diagnosis and monitoring of the progression of the disease [11]. Transcranial magnetic stimulation (TMS) technique can detect cortical hyperexcitability at early stages of the disease, which has been correlated with upper motor neuron dysfunction [12]. On the other hand, the course of ALS can be monitored by assessing lower motor neuron dysfunction using electrical impedance myography, axonal excitability testing, the motor unit number index and muscle ultrasonography [12][13][14]. However, despite the potential neurophysiological biomarkers found so far in assessing progression and early diagnosis, further studies should be conducted involving larger ALS cohorts [12].

Multiple molecular markers have been described in cerebrospinal fluid (CSF), plasma, serum, and even urine and saliva. Unlike CSF, the other fluids possess the advantage of being easily accessible and do not require invasive methods to obtain them, which is an important feature of the ideal biomarker. For this reason, over the last decades, several studies have been conducted to discover new biomarkers in biofluids that are derived from different pathological mechanisms of ALS [15][16][17][18][19]. The best considered biomarkers candidates are inflammatory molecules, metabolic markers and neurofilaments (NFs) [20][21].

To date, NFs are the most promising biomarkers for ALS. Particularly, both NF heavy chain (NFH) and NF light chain (NFL) levels measured in CSF and blood samples can be used to differentiate ALS patients from healthy subjects and/or other neurological diseases [22]. Furthermore, NFH and NFL levels in CSF were negatively correlated with disease duration, which address NFs as potential biomarkers for both diagnosis and prognosis of ALS [22].

Regarding inflammatory mediators, large panels of cytokines, including numerous interleukins, and immune cells, such as T regulatory cells (Treg), have been identified in CSF, plasma or serum, and have been correlated with faster or slower progression [23][24][25].

3. The Role of The Immune System in ALS

The dysregulation of the immune system in ALS results in increased central and peripheral inflammatory responses [26]. Neuroinflammation is characterized by microglial activation, astrogliosis, infiltration of T lymphocytes and monocytes, and overproduction of inflammatory cytokines [27][28][29]. Both innate and adaptive immune responses are involved in ALS progression and can promote either neuroprotection or neurotoxicity depending on disease

stage, evidencing a dual role of inflammation in ALS [30]. Initially, there is an early anti-inflammatory or neuroprotective phase, where neurotrophic factors and anti-inflammatory cytokines, such as interleukin (IL)-4 and IL-10, are secreted by surrounding astrocytes and M2 microglia. As the disease progresses, the neuroprotective response changes to a cytotoxic phase due to the activation of M1 microglia and the consequent release of toxic factors, including reactive oxygen species (ROS) and pro-inflammatory cytokines, such as IL-1β and tumor necrosis factor (TNF)-α, which causes progressive injury to the MN [31].

Apart from microglia and astrocytes, T lymphocytes of the adaptive immune response also play a relevant role in the neurodegeneration observed in ALS. Especially, CD4+ T helper (Th) lymphocytes, including Th2 lymphocytes and Treg, are found in the early neuroprotective compensatory response; on the contrary, CD8+ T cytotoxic lymphocytes, such as Th1 and Th17 lymphocytes, are observed at later stages of the disease [31][32]. Both types of T lymphocytes have shown a dual role: Th1 and Th17 expression was found elevated in blood from ALS patients [33], whereas an upregulation of Treg in blood was associated with slower progression of the disease [34]. Similarly, increasing levels of Treg in animal models at early stages in the disease prolonged survival [35].

Other cells involved in MN degeneration are monocytes and macrophages. They have been found activated in peripheral blood and in CNS, especially in the spinal cord, due to the existent disruption of the blood-spinal cord barrier, in both ALS patients and murine models [36][37]. In line with this, some components of the complement system, which participate in the recruitment of mononuclear cells and macrophages, have been found elevated in CSF, blood, spinal cord and skeletal muscle from ALS patients or murine models [28][38][39]. However, the role of the complement system in ALS pathogenesis remains controversial due to the diverging results based on the great inter-individual differences during disease progression, although aberrant activation of the complement system is suggested to be involved in the pathophysiology in ALS animal models and patients [38].

More recently, the relevant role of the mutation C9orf72 in myeloid cells opened the door to altered microglial function that can link the connection between autoimmunity and ALS/FTD. Some studies on heterozygous C9orf72 (C9orf72+/–) mice have suggested an altered myeloid cell function and systemic immunity. Accordingly, similar immunological consequences have been observed in ALS patients. Therefore, the loss of function of C9orf72 together with a combination of mutations of ALS/FTD genes could promote neurodegeneration [40]. Finally, the consequence of an altered microglia in both mutant SOD1 mice and patients is an amplified generation of pro-inflammatory cytokines that can trigger TNF-α-mediated apoptosis [41].

Despite the diverse humoral and cellular factors being found to be dysregulated, supporting the evident role of neuroinflammation in ALS pathology, to date it is still unclear how these inflammatory mediators can influence the progression of the disease and how they can be helpful in the diagnosis of ALS.

4. Cytokines as Biomarkers: Main Challenges

Over the last decades, many CSF and blood inflammatory cytokines have been found dysregulated in ALS, supporting the relevant contribution of neuroinflammation in the pathogenic mechanisms leading to motor neuron

degeneration in ALS. However, the results shown are not always consistent in all the studies performed, which hampers the translation of a single cytokine as a biomarker to the clinical practice. As an example, it has been reported that the levels of certain cytokines in plasma from ALS patients were highly variable between the first and the second visit to the clinic, and even they did not show differences with healthy controls in some cases [42], exposing the great heterogeneity of the disease. In an attempt to deal with this issue, some authors have proposed that it would be more appropriate to identify panels of biomarkers, rather than focusing on a single target [43][44]. In this sense, panels of cytokines have been analyzed to help in a more accurate prediction of disease progression [45][46]. However, some authors suggest that these promising multivariate models should also include other clinical parameters, such as ALS type (sporadic or familial), disease stage, anatomical onset of motor neuron impairment and even age and gender [23][47].

Another problem that appears in the searching of circulating cytokines as biomarkers is the influence exerted by the action of environment and other factors surrounding the patient. For instance, the upregulation of some circulating cytokines, such as IL-6, IL-8, IL-10, G-CSF and TNF-α, has been linked to exercise [48], and others, including IL-6 and TNF-α, are elevated in a hypoxia status [49], which is a feature frequently found in ALS patients. In addition, the cytokine levels measured in blood in healthy individuals are not stable markers [50]. Cytokines play a relevant role in the immune system, and alterations in this system due to infections, injuries, tissue trauma or inflammation, which is inherent in ALS, can unbalance the immune system, even more under neurodegenerative conditions. This imbalance can promote an intra-individual variation that could explain the high variability of cytokine levels observed in ALS [26][50]. Furthermore, in the context of ALS and FTD, the cytokine profile in blood is also challenging to interpret due to the disease state, environmental factors, and genetic background of the individuals that can lead to controversial results [26].

Therefore, the consideration of circulating cytokines and other circulating targets as biomarkers is being increasingly questioned, mainly due to the opposing and irreproducible results that have been shown in different studies [51]. Additionally, the underlying causes, such as lack of sensitivity, unsuitable normalization or variations in sample handling, together with the difficulties in cytokine assays that are not performed in routine clinical methodology, establish this issue as a real challenge. In an attempt to address this problem, Otto et al. proposed a roadmap for biomarker discovery and provided standard operating procedures that could allow multicenter collaboration and validation of the neurochemical markers discovered in ALS to facilitate their translation to the clinic [52]. In this line, multicenter studies can also shed light on this controversial issue by confirming the results among different centers in the world, as demonstrated in several multicenter studies performed with ALS patients [53][54][55]. Accordingly, it could be interesting to contemplate multicenter studies regarding the most promising inflammatory mediators, which could be helpful to validate them.

References

1. Martin, S.; Al Khleifat, A.; Al-Chalabi, A. What causes amyotrophic lateral sclerosis? F1000Res 2017, 6, 371.
2. Gurney, M.E.; Pu, H.; Chiu, A.Y.; Dal Canto, M.C.; Polchow, C.Y.; Alexander, D.D.; Caliendo, J.; Hentati, A.; Kwon, Y.W.; Deng, H.X. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 1994, 264, 1772–1775.
3. Turner, M.R.; Kiernan, M.C.; Leigh, P.N.; Talbot, K. Biomarkers in amyotrophic lateral sclerosis. Lancet Neurol. 2009, 8, 94–109.
4. Verber, N.S.; Shepheard, S.R.; Sassani, M.; McDonough, H.E.; Moore, S.A.; Alix, J.J.P.; Wilkinson, I.D.; Jenkins, T.M.; Shaw, P.J. Biomarkers in Motor Neuron Disease: A State of the Art Review. Front. Neurol. 2019, 10, 291.
5. Menke, R.A.; Agosta, F.; Grosskreutz, J.; Filippi, M.; Turner, M.R. Neuroimaging Endpoints in Amyotrophic Lateral Sclerosis. Neurotherapeutics 2017, 14, 11–23.
6. Mazon, M.; Vazquez Costa, J.F.; Ten-Esteve, A.; Marti-Bonmati, L. Imaging Biomarkers for the Diagnosis and Prognosis of Neurodegenerative Diseases. The Example of Amyotrophic Lateral Sclerosis. Front. Neurosci. 2018, 12, 784.
7. Simon, N.G.; Turner, M.R.; Vucic, S.; Al-Chalabi, A.; Shefner, J.; Lomen-Hoerth, C.; Kiernan, M.C. Quantifying disease progression in amyotrophic lateral sclerosis. Ann. Neurol. 2014, 76, 643–657.
8. Ferraro, P.M.; Agosta, F.; Riva, N.; Copetti, M.; Spinelli, E.G.; Falzone, Y.; Soraru, G.; Comi, G.; Chio, A.; Filippi, M. Multimodal structural MRI in the diagnosis of motor neuron diseases. Neuroimage Clin. 2017, 16, 240–247.
9. Barritt, A.W.; Gabel, M.C.; Cercignani, M.; Leigh, P.N. Emerging Magnetic Resonance Imaging Techniques and Analysis Methods in Amyotrophic Lateral Sclerosis. Front. Neurol. 2018, 9, 1065.
10. Steinbach, R.; Gaur, N.; Stubendorff, B.; Witte, O.W.; Grosskreutz, J. Developing a Neuroimaging Biomarker for Amyotrophic Lateral Sclerosis: Multi-Center Data Sharing and the Road to a “Global Cohort”. Front. Neurol. 2018, 9, 1055.
11. Huynh, W.; Dharmadasa, T.; Vucic, S.; Kiernan, M.C. Functional Biomarkers for Amyotrophic Lateral Sclerosis. Front. Neurol. 2019, 9, 1141.
12. Vucic, S.; Rutkove, S.B. Neurophysiological biomarkers in amyotrophic lateral sclerosis. Curr. Opin. Neurol. 2018, 31, 640–647.
13. Martinez-Paya, J.J.; Rios-Diaz, J.; Medina-Mirapeix, F.; Vazquez-Costa, J.F.; Del Bano-Aledo, M.E. Monitoring Progression of Amyotrophic Lateral Sclerosis Using Ultrasound Morpho-Textural Muscle Biomarkers: A Pilot Study. Ultrasound Med. Biol. 2018, 44, 102–109.
14. Rios-Diaz, J.; Del Bano-Aledo, M.E.; Tembl-Ferrairo, J.I.; Chumillas, M.J.; Vazquez-Costa, J.F.; Martinez-Paya, J.J. Quantitative neuromuscular ultrasound analysis as biomarkers in amyotrophic

lateral sclerosis. Eur. Radiol. 2019.

15. Tarasiuk, J.; Kulakowska, A.; Drozdowski, W.; Kornhuber, J.; Lewczuk, P. CSF markers in amyotrophic lateral sclerosis. J. Neural Transm. (Vienna) 2012, 119, 747–757.
16. Vu, L.T.; Bowser, R. Fluid-Based Biomarkers for Amyotrophic Lateral Sclerosis. Neurotherapeutics 2017, 14, 119–134.
17. Majumder, V.; Gregory, J.M.; Barria, M.A.; Green, A.; Pal, S. TDP-43 as a potential biomarker for amyotrophic lateral sclerosis: A systematic review and meta-analysis. BMC Neurol. 2018, 18, 90.
18. Bjornevik, K.; Zhang, Z.; O'Reilly, E.J.; Berry, J.D.; Clish, C.B.; Deik, A.; Jeanfavre, S.; Kato, I.; Kelly, R.S.; Kolonel, L.N.; et al. Prediagnostic plasma metabolomics and the risk of amyotrophic lateral sclerosis. Neurology 2019.
19. Calvo, A.C.; Atencia Cibreiro, G.; Torre Merino, P.; Roy, J.F.; Galiana, A.; Juárez Rufián, A.; Cano, J.M.; Martín, M.A.; Moreno, L.; Larrodé, P.; et al. Collagen XIX Alpha 1 Improves Prognosis in Amyotrophic Lateral Sclerosis. Aging Dis. 2019, 10, 278.
20. Costa, J.; de Carvalho, M. Emerging molecular biomarker targets for amyotrophic lateral sclerosis. Clin. Chim. Acta 2016, 455.
21. Floeter, M.K.; Gendron, T.F. Biomarkers for Amyotrophic Lateral Sclerosis and Frontotemporal Dementia Associated With Hexanucleotide Expansion Mutations in C9orf72. Front. Neurol. 2018, 9, 1063.
22. Xu, Z.; Henderson, R.D.; David, M.; McCombe, P.A. Neurofilaments as Biomarkers for Amyotrophic Lateral Sclerosis: A Systematic Review and Meta-Analysis. PLoS ONE 2016, 11, e0164625.
23. Lu, C.H.; Allen, K.; Oei, F.; Leoni, E.; Kuhle, J.; Tree, T.; Fratta, P.; Sharma, N.; Sidle, K.; Howard, R.; et al. Systemic inflammatory response and neuromuscular involvement in amyotrophic lateral sclerosis. Neurol. Neuroimmunol. Neuroinflamm. 2016, 3, e244.
24. Beers, D.R.; Zhao, W.; Wang, J.; Zhang, X.; Wen, S.; Neal, D.; Thonhoff, J.R.; Alsuliman, A.S.; Shpall, E.J.; Rezvani, K.; et al. ALS patients' regulatory T lymphocytes are dysfunctional, and correlate with disease progression rate and severity. JCI Insight 2017, 2, e89530.
25. Michaelson, N.; Facciponte, D.; Bradley, W.; Stommel, E. Cytokine expression levels in ALS: A potential link between inflammation and BMAA-triggered protein misfolding. Cytokine Growth Factor Rev. 2017, 37, 81–88.
26. McCauley, M.E.; Baloh, R.H. Inflammation in ALS/FTD pathogenesis. Acta Neuropathol. 2018.
27. Ransohoff, R.M. How neuroinflammation contributes to neurodegeneration. Science 2016, 353, 777–783.

28. Liu, J.; Wang, F. Role of Neuroinflammation in Amyotrophic Lateral Sclerosis: Cellular Mechanisms and Therapeutic Implications. Front. Immunol. 2017, 8, 1005.
29. Beers, D.R.; Appel, S.H. Immune dysregulation in amyotrophic lateral sclerosis: Mechanisms and emerging therapies. Lancet Neurol. 2019, 18, 211–220.
30. Zhao, W.; Beers, D.R.; Appel, S.H. Immune-mediated mechanisms in the pathoprogression of amyotrophic lateral sclerosis. J. Neuroimmune Pharmacol. 2013, 8, 888–899.
31. Hooten, K.G.; Beers, D.R.; Zhao, W.; Appel, S.H. Protective and Toxic Neuroinflammation in Amyotrophic Lateral Sclerosis. Neurotherapeutics 2015, 12, 364–375.
32. Murdock, B.J.; Bender, D.E.; Segal, B.M.; Feldman, E.L. The dual roles of immunity in ALS: Injury overrides protection. Neurobiol. Dis. 2015, 77, 1–12.
33. Saresella, M.; Piancone, F.; Tortorella, P.; Marventano, I.; Gatti, A.; Caputo, D.; Lunetta, C.; Corbo, M.; Rovaris, M.; Clerici, M. T helper-17 activation dominates the immunologic milieu of both amyotrophic lateral sclerosis and progressive multiple sclerosis. Clin. Immunol. 2013, 148, 79–88.
34. Henkel, J.S.; Beers, D.R.; Wen, S.; Rivera, A.L.; Toennis, K.M.; Appel, J.E.; Zhao, W.; Moore, D.H.; Powell, S.Z.; Appel, S.H. Regulatory T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival. EMBO Mol. Med. 2013, 5, 64–79.
35. Beers, D.R.; Henkel, J.S.; Zhao, W.; Wang, J.; Huang, A.; Wen, S.; Liao, B.; Appel, S.H. Endogenous regulatory T lymphocytes ameliorate amyotrophic lateral sclerosis in mice and correlate with disease progression in patients with amyotrophic lateral sclerosis. Brain 2011, 134, 1293–1314.
36. Zondler, L.; Muller, K.; Khalaji, S.; Bliederhauser, C.; Ruf, W.P.; Grozdanov, V.; Thiemann, M.; Fundel-Clemes, K.; Freischmidt, A.; Holzmann, K.; et al. Peripheral monocytes are functionally altered and invade the CNS in ALS patients. Acta Neuropathol. 2016, 132, 391–411.
37. Gasco, S.; Zaragoza, P.; Garcia-Redondo, A.; Calvo, A.C.; Osta, R. Inflammatory and non-inflammatory monocytes as novel prognostic biomarkers of survival in SOD1G93A mouse model of Amyotrophic Lateral Sclerosis. PLoS ONE 2017, 12, e0184626.
38. Kjaeldgaard, A.L.; Pilely, K.; Olsen, K.S.; Pedersen, S.W.; Lauritsen, A.O.; Moller, K.; Garred, P. Amyotrophic lateral sclerosis: The complement and inflammatory hypothesis. Mol. Immunol. 2018, 102, 14–25.
39. Parker, S.E.; Hanton, A.M.; Stefanou, S.N.; Noakes, P.G.; Woodruff, T.M.; Lee, J.D. Revisiting the role of the innate immune complement system in ALS. Neurobiol. Dis. 2019, 127, 223–232.
40. Lall, D.; Baloh, R.H. Microglia and C9orf72 in neuroinflammation and ALS and frontotemporal dementia. J. Clin. Invest. 2017, 127, 3250–3258.

41. Glass, C.K.; Saijo, K.; Winner, B.; Marchetto, M.C.; Gage, F.H. Mechanisms underlying inflammation in neurodegeneration. Cell 2010, 140, 918–934.
42. Ehrhart, J.; Smith, A.J.; Kuzmin-Nichols, N.; Zesiewicz, T.A.; Jahan, I.; Shytle, R.D.; Kim, S.H.; Sanberg, C.D.; Vu, T.H.; Gooch, C.L.; et al. Humoral factors in ALS patients during disease progression. J. Neuroinflamm. 2015, 12, 127.
43. von Neuhoff, N.; Oumeraci, T.; Wolf, T.; Kollewe, K.; Bewerunge, P.; Neumann, B.; Brors, B.; Bufler, J.; Wurster, U.; Schlegelberger, B.; et al. Monitoring CSF proteome alterations in amyotrophic lateral sclerosis: Obstacles and perspectives in translating a novel marker panel to the clinic. PLoS ONE 2012, 7, e44401.
44. Robelin, L.; Gonzalez De Aguilar, J.L. Blood biomarkers for amyotrophic lateral sclerosis: Myth or reality? Biomed. Res. Int. 2014, 2014, 525097.
45. Su, X.W.; Simmons, Z.; Mitchell, R.M.; Kong, L.; Stephens, H.E.; Connor, J.R. Biomarker-based predictive models for prognosis in amyotrophic lateral sclerosis. JAMA Neurol. 2013, 70, 1505–1511.
46. Martinez, H.R.; Escamilla-Ocanas, C.E.; Tenorio-Pedraza, J.M.; Gomez-Almaguer, D.; Jaime-Perez, J.C.; Olguin-Ramirez, L.A.; Salazar-Marioni, S.; Gonzalez-Garza, M.T. Altered CSF cytokine network in amyotrophic lateral sclerosis patients: A pathway-based statistical analysis. Cytokine 2017, 90, 1–5.
47. Garbuzova-Davis, S.; Ehrhart, J.; Sanberg, P.R.; Borlongan, C.V. Potential Role of Humoral IL-6 Cytokine in Mediating Pro-Inflammatory Endothelial Cell Response in Amyotrophic Lateral Sclerosis. Int. J. Mol. Sci. 2018, 19, 423.
48. Peake, J.M.; Della Gatta, P.; Suzuki, K.; Nieman, D.C. Cytokine expression and secretion by skeletal muscle cells: Regulatory mechanisms and exercise effects. Exerc. Immunol. Rev. 2015, 21, 8–25.
49. Moreau, C.; Devos, D.; Brunaud-Danel, V.; Defebvre, L.; Perez, T.; Destee, A.; Tonnel, A.B.; Lassalle, P.; Just, N. Elevated IL-6 and TNF-alpha levels in patients with ALS: Inflammation or hypoxia? Neurology 2005, 65, 1958–1960.
50. Selmaoui, B.; Sackett-Lundeen, L.; Haus, E.; Touitou, Y. Large intra-individual variability of plasma cytokines in healthy young men: A two 24-h study over a month. Biol. Rhythm Res. 2016, 47, 267–273.
51. Barschke, P.; Oeckl, P.; Steinacker, P.; Ludolph, A.; Otto, M. Proteomic studies in the discovery of cerebrospinal fluid biomarkers for amyotrophic lateral sclerosis. Expert Rev. Proteomics 2017, 14, 769–777.
52. Otto, M.; Bowser, R.; Turner, M.; Berry, J.; Brettschneider, J.; Connor, J.; Costa, J.; Cudkowicz, M.; Glass, J.; Jahn, O.; et al. Volcano Group Roadmap and standard operating procedures for

biobanking and discovery of neurochemical markers in ALS. Amyotroph. Lateral Scler. 2012, 13, 1–10.

53. Mitsumoto, H.; Factor-Litvak, P.; Andrews, H.; Goetz, R.R.; Andrews, L.; Rabkin, J.G.; McElhiney, M.; Nieves, J.; Santella, R.M.; Murphy, J.; et al. ALS COSMOS Study Group ALS Multicenter Cohort Study of Oxidative Stress (ALS COSMOS): Study methodology, recruitment, and baseline demographic and disease characteristics. Amyotroph. Lateral Scler. Frontotemporal Degener. 2014, 15, 192–203.
54. Calvo, A.; Moglia, C.; Lunetta, C.; Marinou, K.; Ticozzi, N.; Ferrante, G.D.; Scialo, C.; Soraru, G.; Trojsi, F.; Conte, A.; et al. Factors predicting survival in ALS: A multicenter Italian study. J. Neurol. 2017, 264, 54–63.
55. D'hulst, L.; Van Weehaeghe, D.; Chio, A.; Calvo, A.; Moglia, C.; Canosa, A.; Cistaro, A.; Willekens, S.M.; De Vocht, J.; Van Damme, P.; et al. Multicenter validation of -FDG PET and support-vector machine discriminant analysis in automatically classifying patients with amyotrophic lateral sclerosis versus controls. Amyotroph. Lateral Scler. Frontotemporal Degener. 2018, 19, 570–577.