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Panagiotis Loupis, PhD-student

Supervisor: Dr. Ieronymos Zoidakis

Biomedical Research Foundation, Academy of Athens,
Department of Biotechnology, Soranou Efessiou 4,
11527 Athens, Greece

Validation of novel protein biomarkers in body fluids of patients with Multiple Sclerosis

Multiple Sclerosis (MS) is an autoimmune disease with a complex pathogenesis that is driven by inflammation and axon degeneration, due to inflammatory cells and mediators accumulation in white matter. Inflammatory infiltrates include CD4 and CD8 T cells, activated monocytes, and B cells are present within lesions in the CNS resulting in degradation of the myelin sheath surrounding nerves [1, 2]. MS is classified into the four following subtypes: (1) primary progressive MS (PPMS) exhibits gradual, continuously increasing symptoms with minor fluctuations; (2) relapsing-remitting MS (RRMS) is characterized by episodes of diverse neurological dysfunction followed by periodic remissions and a variable degree of recovery; (3) secondary-progressive MS (SPMS) initially presents as RRMS but progresses to a steady worsening of clinical symptoms during the progressive phase; (4) progressive-relapsing MS (PRMS) is characterized by distinct relapsing-remitting periods with progressive chronic worsening between periods[3]. Clinical symptoms vary depending on the lesion burden and location and include muscle weakness, blurred vision, dizziness, fatigue, and balance and walking problems. Thus, the clinical phenotype of MS is very heterogeneous and the course of the disease is difficult to predict. Neither the frequency of relapses (disease activity) nor the accumulated disability represents an accurate predictor of disease outcome[4].

There has been considerable interest in finding biomarkers in MS over the last two decades. However, with some exceptions, many suggested biomarkers are correlative and have yet to be shown to have useful prognostic capability. Consequently, using a biomarker for one disease type may not be useful for a different type and so the identification of predictive biomarkers in MS remains a critical and open question in the field.

Cerebrospinal fluid (CSF) and blood have long been investigated as sources of accessible, dynamic and cost-effective biomarkers of MS that could, in addition to MRI shed light on the ongoing pathological mechanisms. Furthermore, with the advances in ‘omics’ technologies, we now have the tools to systematically screen body fluids for novel biomarkers for different clinical purposes [5, 6]. The ‘omics’ approach offers a shift from the old, hypothesis-based single-marker paradigm, towards compilation of panels of multiple biomarkers that reflect multiple disease mechanisms[3].

Proteomics hold promise as a noninvasive, high-throughput means of analysis of biomarkers, capable of detecting early stage disease, prognosis, but also monitoring

treatment efficacy and assisting in the development of novel targeted therapeutics. On this project, we use targeted proteomics in order to investigate differences in the concentration of the selected proteins, among the two groups of cases and controls. This selection was based on literature mining for proteins that take part in the pathophysiology of the disease, then we concluded to a list of the most promising biomarkers, to assist in stratification of patients subtypes and early prognosis of the disease. The literature mining was concentrated on proteins involve in immune regulation mechanisms, oxidative stress and neurodegenerative activities. Our list of proteins includes interleukins (IL-6, IL-10, IL-12, IL-17A, IL-23), interferons (INF-γ, IFN-β), other cytokines (TNF-a, OPN, CXCL-13) and the collagen degrading enzyme MMP-9.

The method we intend to create and optimize is Multiple Reaction Monitoring (MRM) in a triple quadrupole mass spectrometer, in order to conduct measurements across multiple samples, with high reproducibility and precision. This allows concurrent quantification of multiple analytes. Our objective is to detect differences in the concentration of the proteins among the two groups (cases vs controls) and subsequently, variations between the different subtypes of the disease. In order to enhance the reliability of our results, we will validate our measurements with ELISA. This technique is very sensitive and currently used in the clinical setting, with the ability to detect proteins on body fluids even in very low concentrations (pg/ml). Thus, we plan to generate correlation graphs for establishing the concordance between MRM and ELISA values. Therefore, we consider that the ELISA sets for IL-6, IL-10, IL-12p40, IL-17A, INF-gamma and TNF-alpha will be valuable tools to determine the concentration of these low abundance proteins in CSF and plasma samples. The expected results have the potential for clinical application and could improve the management of MS patients.

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