

## ABSTRACT

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Multiple sclerosis (MS) is a chronic neurodegenerative disease characterized by an autoimmune background. The condition primarily affects the central nervous system (CNS), leading to focal and disseminated lesions in the brain and spinal cord. The development of MS is driven by a persistent inflammatory response involving autoreactive T and B-cells directed against myelin antigens. The inflammatory process in the CNS is exacerbated by increased permeability of a disrupted blood-brain barrier (BBB), which is why the pathophysiology of MS involves not only neuroinflammatory but also vascular factors.

MS is a heterogeneous disease, difficult to categorize, with a varied course and variable phenotype. The most common form of the disease is the inflammatory relapsing-remitting MS (RRMS), whereas the secondary progressive MS (SPMS), which typically develops after RRMS, is characterized by progressive neurodegenerative processes without periods of relapses and remissions. However, recent reports indicate the coexistence of inflammatory and neurodegenerative features in both phenotypes, which complicates early differentiation, therapeutic decision-making, and prognosis of disease progression.

The primary objective of the research undertaken in this doctoral dissertation was to identify markers enabling the differentiation of disease phenotypes in patients with RRMS in remission and SPMS. In the first stage of the study platelet-leukocyte hetero-aggregates (PLAs) were characterized as elements linking vascular injury pathogenesis with inflammatory process development. Using migration assays, microscopic imaging, and flow cytometry, increased leukocyte chemotaxis toward platelets and the formation of PLAs, predominantly involving B-cells, were demonstrated in MS. Moreover, a potential role of the CD40-CD40L axis in the formation of PLAs was identified, with a significant correlation between platelet CD40L and lymphocyte CD40, the strongest in the analysis of co-expression of these antigens on platelets and B-cells in SPMS.

In the second stage of the study a screening analysis of the differential expression of microRNA (miRNA) originating from extracellular vesicles (EVs) using RNA sequencing was conducted, followed by a validation of the results using RT-qPCR, determination of the concentration of plasma inflammatory cytokines and markers of neuronal/glial damage using the Bio-Plex system and ELISA technique, respectively. This was followed by an integrative bioinformatic analysis of the results. Four miRNAs differentiating RRMS and SPMS were identified (miR-760, miR-98-5p, miR-301a-3p, miR-223-3p), with miR-760 emerging as the strongest single predictor of classification into

the RRMS phenotype. In SPMS, specific correlations were observed between miR-760 and both interleukin (IL) 4 and IL-17, as well as between miR-98-5p and IL-17. A model combining miRNA expression with the level of basic fibroblast growth factor (FGF basic) achieved an AUC of 0.97 (sensitivity 93.3%, specificity 90%), confirming its high ability to discriminate RRMS vs SPMS.

In the third stage, EVs presenting the L1 cell adhesion molecule (L1CAM) in serum and cerebrospinal fluid (CSF) were comprehensively characterized. L1CAM<sup>+</sup> is a protein used to enrich the neuronal fraction of EVs during their isolation. The studies were conducted in terms of vesicle size, concentration, morphology, protein cargo, and surface antigens phenotype. Analyses evaluated the usefulness of L1CAM<sup>+</sup> EVs as dynamic biomarkers for monitoring response to rituximab treatment, an anti-CD20 monoclonal antibody, in RRMS patients. On this basis, different immune profiles were obtained before and after therapy, highlighting the potential of L1CAM<sup>+</sup> EVs in monitoring immunosuppressive treatment targeting B-cell depletion.

In summary, the research conducted in this doctoral thesis expands our understanding of the pathophysiological mechanisms of MS, linking the lymphocyte-dependent immune response with vascular injury. Furthermore, the study identifies opportunities for the use of potential non-invasive biomarkers to differentiate RRMS and SPMS, as well as and to monitor CNS immunopathology and treatment response.

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