Title: **Alternative spliced genes in peripheral blood mononuclear cells of MS patients**

Short title: NLRP3 inflammasome alternative spliced mRNA

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The combination of membrane proteins and their soluble isoforms, originated by alternative splicing, is an important source for the search of non-invasive biomarkers in MS. The aim of this study was to identify mRNA transcriptomic isoforms associated with the different clinical forms of MS, and to establish whether a differential expression can provide insight into some of the mechanisms underlying the pathogenesis of the different forms. 1475 unregulated isoforms corresponding to 960 genes were reported in an RNAseq analysis of 19 MS patients (7 RRMS, 7 SPMS and 5 PPMS) and 9 controls (7 healthy controls (HC) as well as 2 patients with other neurological inflammatory diseases). The majority of these genes are involved in the GO biological processes of defense response, cytokine mediated signaling pathway, inflammatory response and cell surface receptor signaling pathway. Of particular interest resulted the NLRP3 inflammasome-signaling pathway, where over 40 isoforms were found overexpressed.

A validation study with 78 MS patients (45 RRMS and 33 SPMS) and 41 HC matched in aggregate by age and gender, was performed by PCR. Primers were design for 30 isoforms, 26 of them are components of the NLRP3 inflammasome pathway signalling. The results show that IL-1β-206 ($p=0.0056$), IL-1β-207 ($p=0.0071$) and IL-1β-208 ($p=0.0016$ are downregulated in patients with MS, in a Mann-Whitney test, whereas IL1RN-205 ($p=0.0022$) and NF-kBIA-206 ($p<0.0001$) are overexpressed in MS when compared to HC. In the comparison between the different clinical forms, IL1RN-205 (p adj. <0.05), NF-kBIA-206 (p adj.<0.05) and NF-kBIZ-206 (p adj.<0.01) were found to be overexpressed in RRMS over HC.

Interestingly, NF-kB2-203 ($p<0.01$) and NF-kBIZ-206 ($p<0.001$) are differently expressed between RRMS and SPMS. The combination of these isoforms could be a good potential biomarker that, if confirmed, could help in the diagnosis of RRMS and progression to the clinical form, SPMS.