Proteomic analysis of CNS-derived Micro Vescicles in the Cerebrospinal Fluid of Multipls Sclerosis patients

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Introduction. Multiple Sclerosis (MS) is characterized by a diffuse inflammation, which involves both grey and white matter of the Central Nervous System (CNS). While higher concentrations of extracellular microvescicles (MVs) have been demonstrated in the cerebrospinal fluid (CSF) of MS patients, their role in the immune-pathogenesis of the disease is still unclear.

Aims. To perform the proteomic analysis of MVs derived from the CSF of MS patients.

Materials and Methods. CNS-derived MVs were purified from the CSF of 3 patients with early-onset Relapsing Remitting Multiple Sclerosis, 3 patients with Clinically Isolated Syndrome (CIS) and one subject with unspecific white matter abnormalities. The diagnosis was achieved in agreement with the McDonald 2010 criteria. CSF specimens were analyzed by "proteomic phenotyping" approach. The proteomic analysis of CNS-derived MVs was performed using nanocapillary liquid chromatography-tandem mass spectrometry (nano-LC-MS/MS). Protein MVs were separated by SDS-PAGE and the proteins were digested in-gel with trypsin to obtain peptides that were fragmented using nano-LC-MS/MS. All MS/MS data were searched against a human protein database downloaded from the NCBI database (www.ncbi.nlm.nih.gov). The classification of MVs protein content was based on Gene Ontology for cellular localization and biological process, using DAVID and GORILLA softwares.

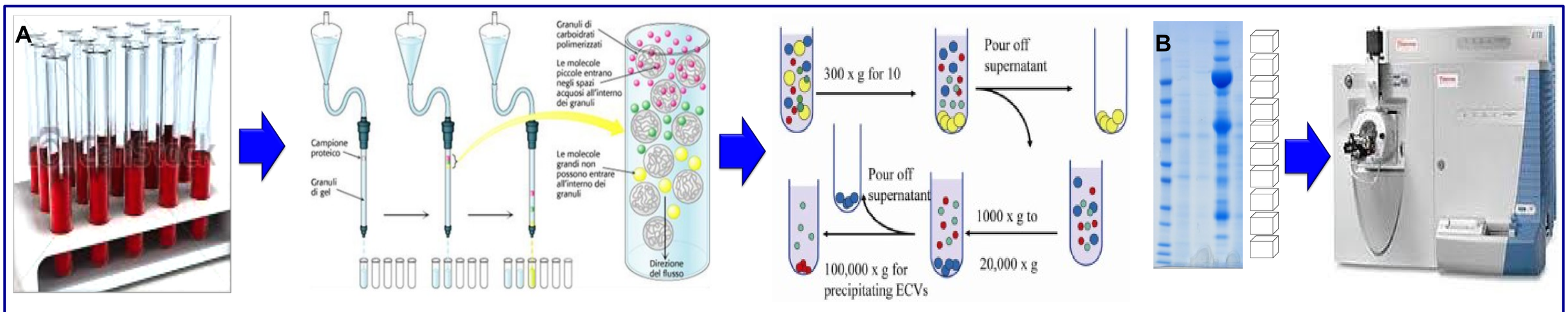

Figure 1. Materials and Methods.
Purification of blood-derived MVs by size-exclusion chromatography from plasma of RRMS patients (A). Proteomic analyses of purified blood-derived MVs were carried out through pre-fractionation of MV protein samples by one dimensional SDS-PAGE followed by LC-MS/MS using an LTQ-XL instrument (Thermo, B).

Results. Proteins identified by proteomic analysis (Table1) showed a significant enrichment of GO-terms for extracellular vesicles-associated proteins (GORILLA software) exclusively in MS patients (Figure 2). Moreover, DAVID software disclosed that 93 out of 115 proteins (80.9%) were extracellular MVs proteins, while and 20 out of total 115 proteins (17,4%) were associated with myelin sheath.

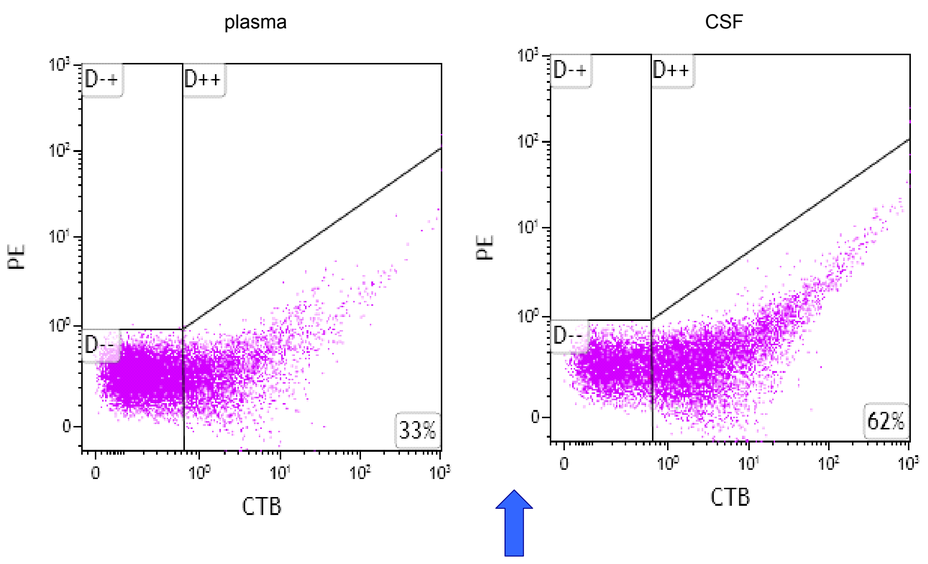

Figure 2. In a MS patient, CSF-MV detection was higher than in a CIS patient.

Table1. Within MVS, few CNS- specificproteins were detected only in RRMS patients

O00533	Neural cell adhesion molecule L1-like protein
Q92823	Neuronal cell adhesion molecule
P30086	Phosphatidylethanolamine-binding protein 1
P68366	Tubulin alpha-4A chain
Q12860	Contactin-1
Q9UQM7	Calcium/calmodulin-dependent protein kinase type II subunit alpha
P13637	Sodium/potassium-transporting ATPase subunit alpha-3
Q96KN2	Beta-Ala-His dipeptidase

Conclusions. We identified a higher concentration of extracellular MVs–related protein, as well as a relevant number of myelin-associated proteins in MS but not in CIS patients. A larger cohort of patients should be studied to identify MV-associated proteins as biomarkers of active inflammation/demyelination.

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