Biomarkers for disease progression: microvesicles in Amyotrophic lateral sclerosis.

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Objectives. Microvesicles (MVs) are membrane vesicles (100-1000 nm), mediator of important physiological and pathological intercellular activities through the transfer of their cargo (proteins, DNA and RNA) between target cells (1). MVs from prion-infected neuronal cells can initiate prion propagation in uninfected cells (2). The aim of this study was to isolate MVs in plasma of Amyotrophic Lateral Sclerosis (ALS) patients and characterize them in order to discover a new mechanism of disease propagation.

Methods. MVs were isolated from plasma of 32 ALS, 32 healthy volunteers and 30 Alzheimer's Disease (AD) patients by ultracentrifugation. SOD1, TDP43, FUS protein level was investigated by WB and normalized against Annexin V. Markers for MVs of leukocyte (CD45), endothelial (CD31), platelet (CD61), erythrocyte (CD235a) derivation and Annexin V were used for flow cytometry. Flow cytometry markers % for ALS patients were classified on their progression rate at baseline (PRB) in slow, intermediate and fast progressing patients (3).

Results. Higher misfolded SOD1 was found in plasma derived MVs of ALS patients compared to controls (two fold more the controls, ANOVA test, p< 0,0001). TDP43 did not show significant difference between control and patients. We further investigated which cell derived MVs were responsible for the transport of misfolded proteins between cells. We first investigated CD45, CD31, CD61, CD235a derived MVs from plasma of ALS, AD and healthy controls by flow cytometry. Interestingly we found a group of slow progressing ALS patients with high expression of CD45+ Annexin V+ MVs compared to another with low expression of CD45+ Annexin V+ MVs (6 fold, p< 0,0001). Patients with high level of CD45 had an increased misfolded SOD1 level in CD45 MVs compared to patients with low level. Further analysis are needed in other ALS patients with fast progression rate to confirm the role of MVs as toxic proteins carrier and as suitable biomarkers in ALS.

Conclusion. Extracellular vesicles have a relevant role in the ALS disease propagation. Leukocyte derived MVs can be overexpressed in a group of ALS patients and they might be the "carriers" of misfolded proteins, main cause of disease propagation.

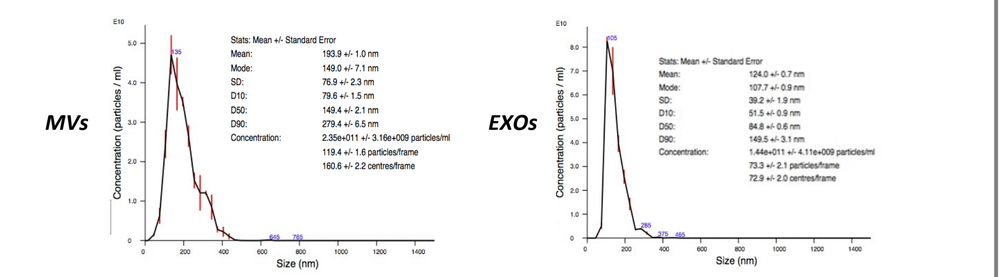

Figure 1. Nanotracking analysis confirmed the purity of MVs included in the range of 100-400 nm in comparison to exosomes (EXOs) in the range of 30-130 nm.

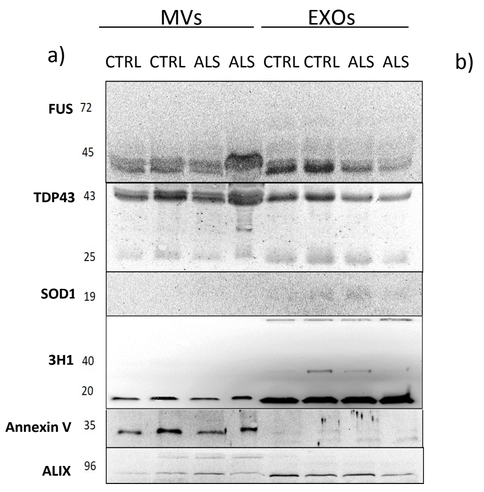

Figure 2. a) WB analysis of FUS, TDP43, SOD1 and misfolded SOD1 (3H1) level in MVs. EXOs were used as control. Annexin V and Alix were used respectively as MVs and EXOs loading controls. b) Densitometric analysis of misfolded SOD1 (3H1) and TDP43 in MVs and EXOs lysate from 20 ALS patients and matched controls (*p<0.05; *** p<0.001).

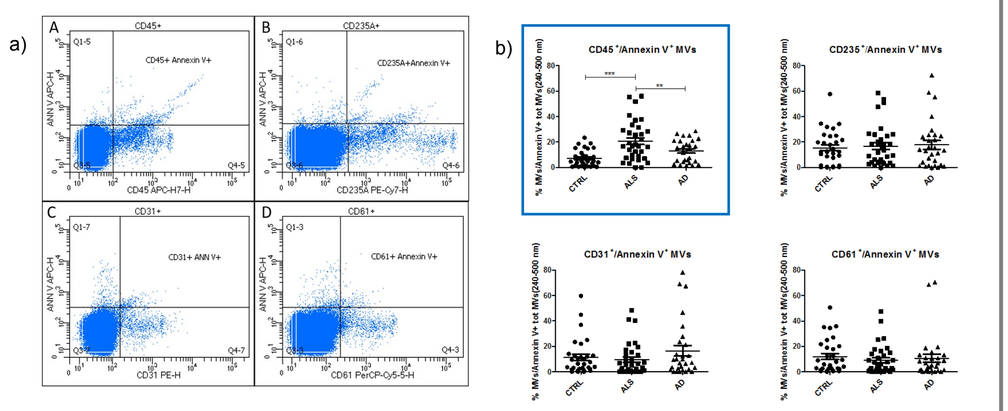

Figure 3. a) Flow cytometry dot plots of MVs, isolated from plasma of an ALS patient labelled with Annexin V and CD45 (A) CD235A (B) CD31 (C) CD61 marker (D).
b) 80% of ALS patients in our cohort presented a higher % Annexin V+ CD45+ MVs in their plasma in comparison to the mean of controls and AD group.

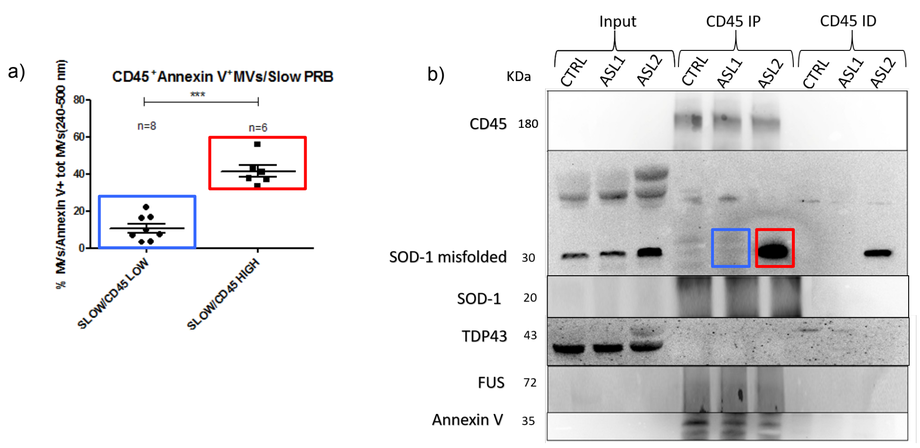

Figure 4. a) Slow PRB (progression rate at baseline) ALS patients could be classified in low CD45+ MVs (n=8) and high CD45+ MVs (n=6) (t test ***p value<0,0001).
B)Immunoprecipitation of plasma derived CD45+ MVs in healthy control and ALS patients. Misfolded SOD1 is highly expressed in CD45+ MVs in SLOW progression/high CD45 ALS in comparison to SLOW progression/low CD45 patient. These data were seen in 14 ALS patients and 8 healthy controls.

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