Leukocyte derived microvesicles correlate with disease progression in ALS

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Objectives. The lack of biomarkers in Amyotrophic Lateral Sclerosis (ALS) makes it difficult to determine the stage of the disease in patients and therefore, it delays therapeutic trials. Blood contains extracellular vesicles, spherical vesicles heterogeneous in size (30 nm - 1 µm in diameter) which are classified mainly, on their biogenesis, dimension and superficial markers, in exosomes (EXOs) and microvesicles (MVs) (1). Misfolded proteins (SOD1, TDP-43) are templates for the formation of protein oligomers that accumulate and interfere with neuronal function, eventually leading to cell death and they can be transported by EVs in human biofluids. The aim of the present study is to characterize MVs and EXOs in plasma of ALS patients, in order to discover a new mechanism in disease progression.

Methods. Microvesicles and exosomes were isolated from plasma of 40 ALS, 28 AD patients and 36 healthy volunteers by ultracentrifugation. Nanoparticle tracking analysis was carried out by Nanosight NS300. Markers for MVs of leukocyte (CD45), endothelial (CD31), platelet (CD61), erythrocyte (CD235a) derivation and Annexin V were used for flow cytometry. SOD1, TDP43 intravescicular protein level was investigated by WB.

Results. Mean dimension resulted increased in ALS patients compared to controls both for microvesicles (t test, $**p<0.01$) and for exosomes (t test, $***p<0.001$) (*Figure 1*). Higher misfolded SOD1 was found in plasma derived EXOs of 30 ALS patients compared to MVs (ANOVA test, $***p< 0.001$), while TDP-43 protein level resulted increased in MVs derived from ALS patients compared to CTRLs (ANOVA test, $*p< 0.05$) (*Figure* 2). Among four different markers detected by flow cytometry, LMVs (leukocyte derived microvesicles-CD45+MVs) were mostly present in 40 ALS patients compared to 28 Alzheimer's Disease (AD) patients and 36 healthy donors (ANOVA test, $**p<0.01$, $***p<0.001$) (*Figure 3A*). The percentage of LMVs was inversely correlated with the progression rate at last visit in fast progressing patients (Spearman r=-0.52, $*p$=0.02) and directly correlated with the progression rate in slow progressing patients (Spearman r =0.38, $*p$= 0.038) (*Figure 3E and 3F*). Isolated LMVs carried misfolded SOD1 and its protein level was strongly associated with the percentage of LMVs in slow progressing patients (Spearman r=0.81, $***p$= 0.001) (*Figure 4*).

Conclusions. Mean dimension resulted increased in ALS patients compared to controls both for microvesicles and for exosomes. There are different misfolded protein cargo between microvesicles and exosomes. Leukocyte derived MVs are regulated by the rate of disease progression in ALS patients and can act as "carriers" of misfolded proteins, main cause of disease propagation. Correlation with the clinical history of patients makes MVs possible biomarkers for the disease.

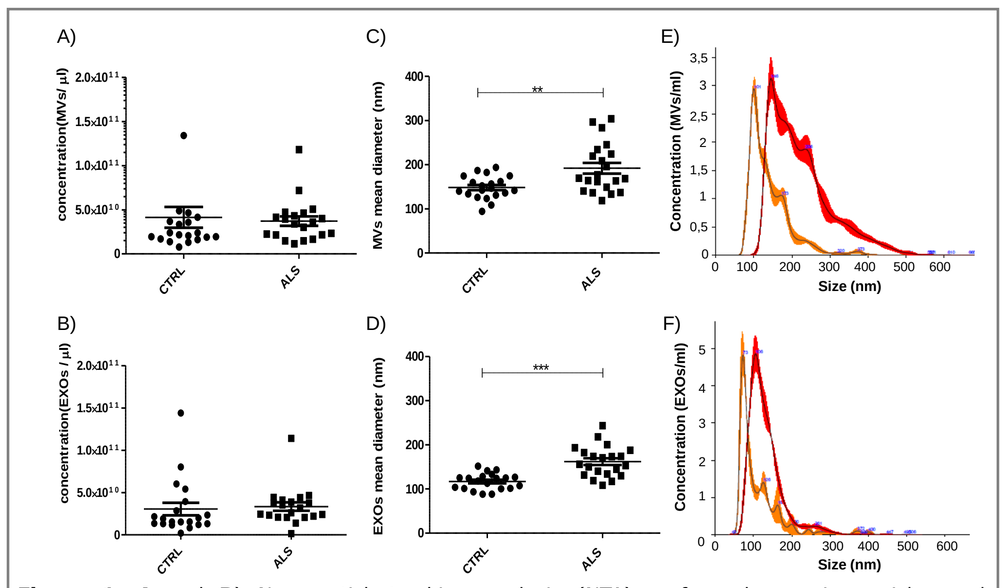

Figure 1. A and **B**) Nanoparticle-tracking analysis (NTA) performed on microvesicles and exosomes concentration did not show any difference between ALS patients and controls (n = 20 for group). **C** and **D**) Mean diameter averaged of microvesicles and of exosomes from ALS patients were significantly higher than healthy donors (CTRL) (t test, $**p<0.01$, $***p<0.001$). **E** and **F**) NTA profiles overlay between ALS MVs (E-red line) and CTRL MVs (E-orange line) and ALS EXOs (F-red line) and CTRL EXOs (F-orange line). The mode of ALS MVs and EXOs were higher than CTRL MVs and EXO.

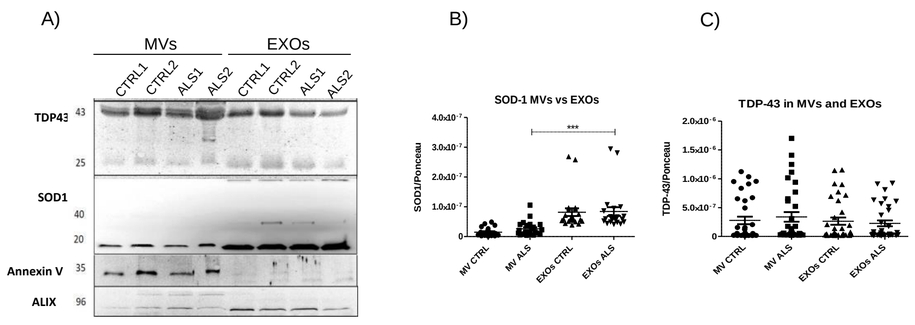

Figure 2. (**A**) Misfolded SOD1, TDP-43 detection by Western Blot analysis in MVs and EXOs derived from plasma of 2 ALS and 2 healthy donors. (**B**) Densitometric analysis of misfolded SOD1 (3H1) and TDP-43 (**C**) in MVs and EXOs lysate from 30 ALS patients and matched controls (*** p<0.001). Higher misfolded SOD1 protein level was found in plasma derived EXOs of ALS patients compared to MVs (ANOVA test, ***p< 0,001), but no difference was detected between MVs and EXOs of CTRLs and ALS. No statistically significant difference was found between MVs and EXOs in TDP-43 content.

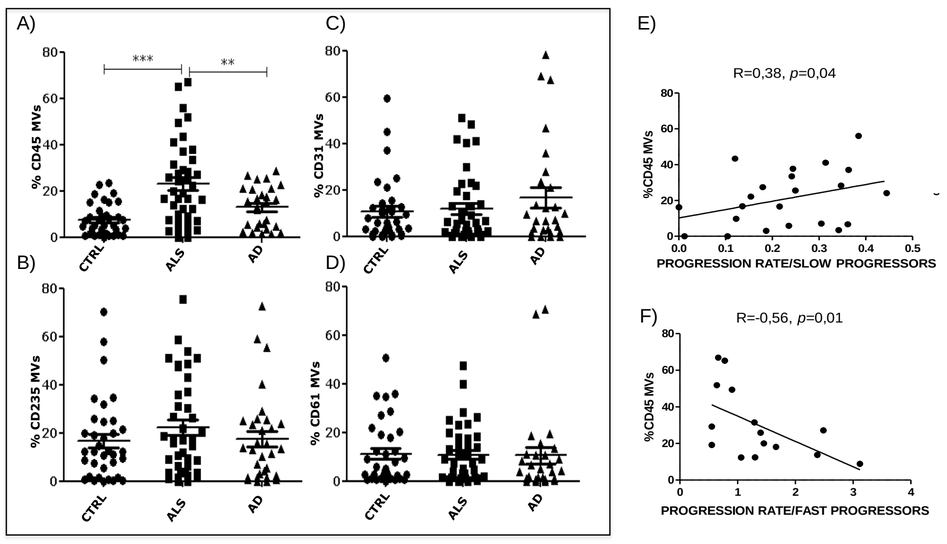

Figure 3. Dot plot of % MVs isolated from plasma of an ALS patient labelled with Annexin V and CD45 (**A**), CD235A (**B**), CD31 (**C**), CD61 (**D**) markers characterized by flow cytometry. **E**) LMVs percentage (CD45 % MVs) was positively correlated to the progression rate in the group of SLOW progressors ALS patients (Spearman test, r=0.372, p=0.04). **F**) Conversely, LMVs percentage (CD45 % MVs) was negatively correlated with the progression rate in the group of FAST progressors ALS patients (Spearman test, r=0.522, p=0.01).

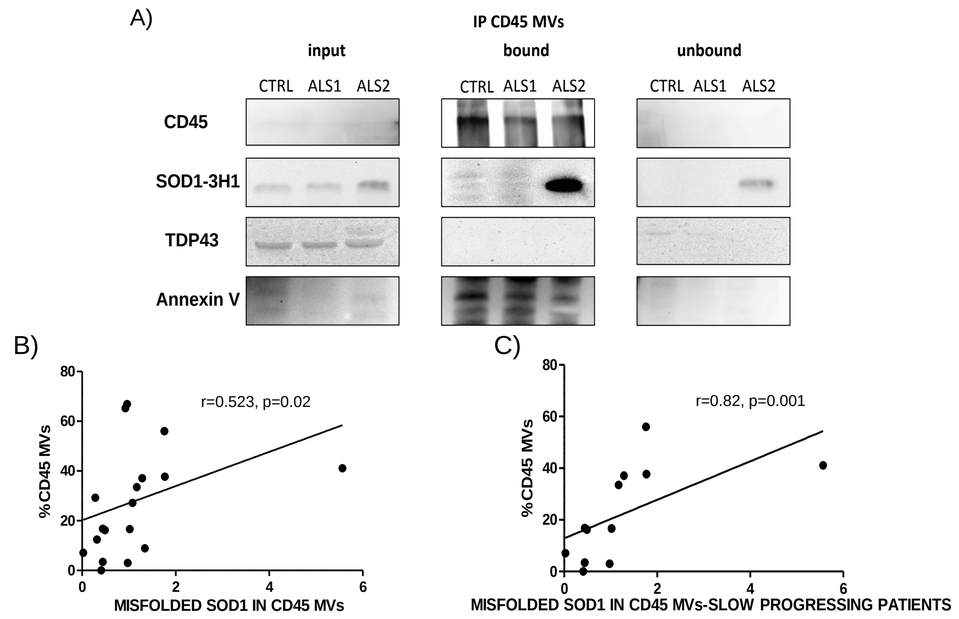

Figure 4. A) Misfolded SOD1 was present in CD45+ MVs from ALS patients and CTRL detected by Western Blot. Input=MVs whole lysate; IP=immunoprecipitated MVs; ID=flow-through; **B**) CD45+ MVs percentage (%) was directly correlated with misfolded SOD1 protein level carried by CD45+MVs in ALS patients (Spearman correlation of r=0.523, p=0.02). **C**) A stronger correlation of CD45+ MVs % with misfolded SOD1 in slow progressing ALS patients was found with r=0.82, p=0.001.

References

1) Cocucci E et al. J., Trends Cel Biol 2015; S0962-8924.
2) Polymenidou M, Cleveland DW, Cell 2011;147:498–508.