Circulating GLAST+ Extracellular Vesicles (EVs) are a new biomarker for Amyotrophic Lateral Sclerosis (ALS)

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INTRODUCTION

- **Amyotrophic lateral sclerosis** (ALS) is a progressive **neurodegenerative disease**, and its pathogenesis is not yet fully understood; no **reliable biomarker** is available neither for ALS diagnosis nor for the monitoring of disease progression.
- Astrocytes are abundant and dynamic glial cells exclusive to the central nervous system (CNS). It has been hypothesized that astrocytes-derived EVs are involved in neuroinflammatory processes. As shown in mice, they express on their **surface GLAST protein**, which is the transporter of glutamate and one of the main neurotransmitter in the CNS.
- In the last years, **extracellular vesicles** (EVs), that are nanoparticles secreted by every cell, emerged **as diagnostic biomarkers for several human diseases. Indeed, they express on their surface markers from the parental cell.** Nevertheless, their application in diagnostic is currently limited by their methods of identification, which are laborious and time consuming. In our laboratory we have established a fast method to detect EVs in liquid biopsy by flow cytometry.

- **The aim of this study was to set a method to identify circulating GLAST+ EVs in ALS patients in order to correlate them with the clinical outcomes.**

METHOD

- We enrolled **55 ALS patients** and **28 healthy age-matched controls (HC)** at the Tertiary ALS Centre in the Maggiore della Carità Hospital of Novara.
- Plasma EVs were isolated by ultracentrifugation (UC) at 100,00xg at 4°C for 1 hour. EVs were analysed by nanoparticle tracking analysis (NTA) (Malvern, Framingham, MA), in order to determine both EVs size and concentration in HC (n=10) and ALS patients (n=12) (Figure 1A).
- **The most abundant circulating EVs** (endothelial-, leukocyte-, and platelet-derived EVs) and GLAST+ EVs were identified in blood and plasma of patients and HC by flow cytometry using a Custom EVs detection kit (Becton and Dickinson). This method is fast (1hr) and is well established in our laboratory (Figures 1B-D) (Cappellano G. et al. Cells. 2021. doi: 10.3390/cells10010085).

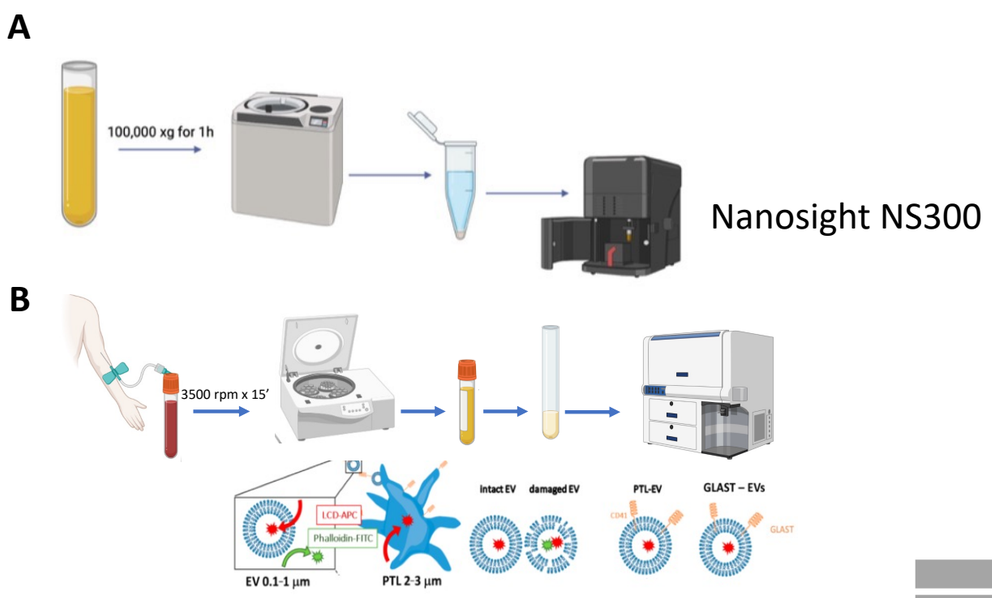

Figure 1. **A-B)** Schematic representation of the typical steps for NTA and flow cytometry analysis of EVs **C)** Gating strategies for circulating EVs and **D)** GLAST+ EVs.

CONCLUSIONS

- We identified for the first time circulating astrocyte derived GLAST+ EV in ALS patients.
- The finding that GLAST+ EVs, released by astrocytes, are increased in ALS patients might suggest their use as a biomarker for ALS. We detected them by applying an easy method which can be translated in clinical practise entering the diagnostic process. These results are preliminary and will be confirmed in a larger cohort of ALS patients.

RESULTS

NTA showed that both **size and concentration** of plasma EVs did not vary between HC and ALS patients (Figure 2).

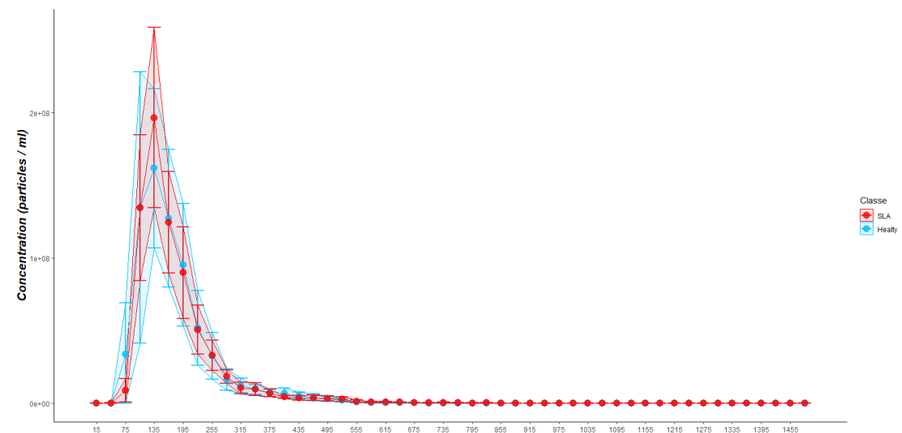

Figure 2. Size (nm) and concentration (EVs/ml) of plasma-derived EVs by NTA.

Flow cytometry analysis of endothelial-, leukocyte- and platelet-derived EVs showed no statistical significant differences between HC and ALS patients (Figure 3).

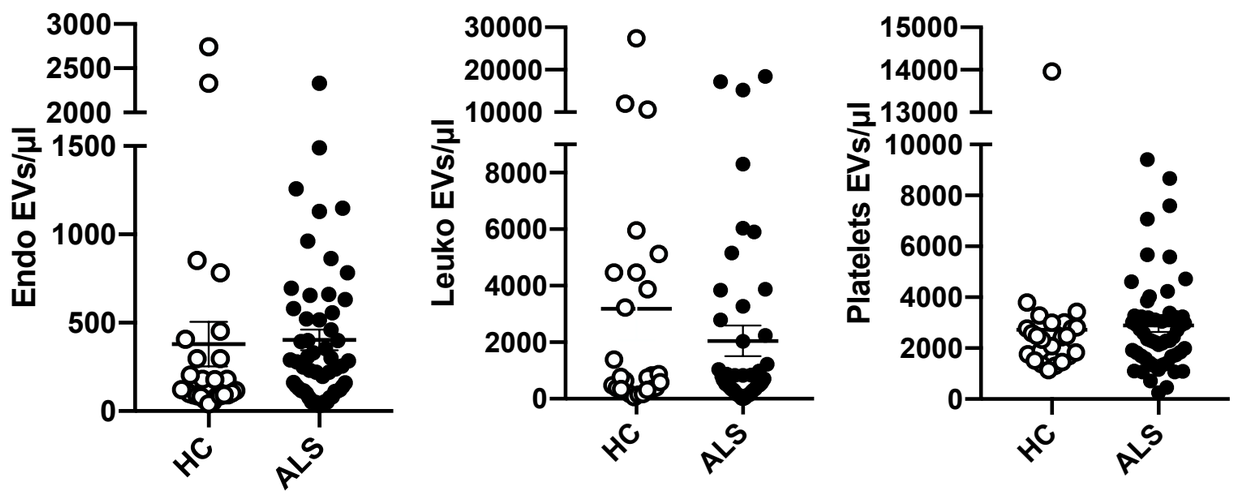

Figure 3. Counts of endothelial- (endo), leukocyte- (leuko), and platelet-derived EVs in HC and ALS patients.

Interestingly, we found that **GLAST+ EV counts were significantly increased in ALS patients compared to HC** (Figure 4). No clinical difference allowed us to associate these EVs with any clinical outcome.

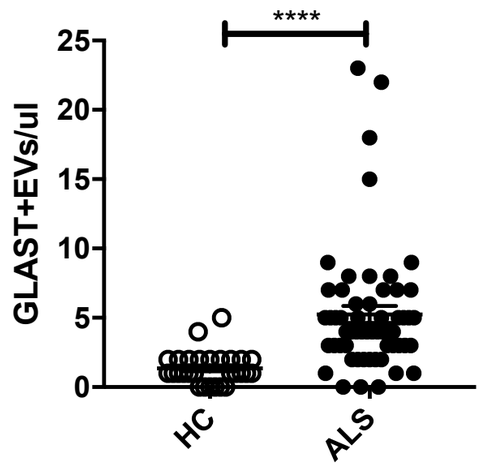

Figure 4. ***A)*** GLAST+ EVs counts are increased in ALS patients.

	Forma di malattia			Mutazione		
	Spinale N=38	Bulbare N=20	p-value	C9Orf72 N=8	No N=49	p-value
Endo EV, mediana (Q1-Q3)	226.09 (98.55;515.94)	289.86 (144.93;605.8)	0.3422	718.84 (120.91;1203.59)	252.17 (115.94;460.32)	0.1833
GLAST EV,mediana (Q1-Q3)	4 (3;5)	4 (3;8)	0.6455	4 (2;7)	4 (3;7)	0.5615
Leuko EV, mediana (Q1-Q3)	524.64 (209.52;2237.68)	460.87 (266.67;1124.7)	0.7416	367.29 (228.99;617.39)	631.88 (324.64;2237.68)	0.3133
PLT EV, mediana (Q1-Q3)	2286.96 (1420.29;3113.04)	2742.03 (1866.67;3252.18)	0.3334	1826.09 (1454.24;3298.55)	2820.29 (1843.48;3229)	0.2662

	Progressione			Quadro congnitivo		
	Lenta N=34	Veloce N=27	p-value	Normale N=41	Alterato N=20	p-value
Endo EV, mediana (Q1-Q3)	243.48 (115.11;430.16)	263.77 (98.55;695.65)	0.6117	255.07 (127.54;521.74)	240.58 (114.29;556.52)	0.8885
GLAST EV,mediana (Q1-Q3)	4 (3;6)	5 (3;7)	0.252	4 (3;5.5)	4 (3;8)	0.7626
Leuko EV, mediana (Q1-Q3)	524.64 (229.4;1626.09)	472.46 (324.64;985.51)	0.972	521.74 (284.06;2794.2)	495.65 (249.28;1217.39)	0.4338
PLT EV, mediana (Q1-Q3)	2168.12 (1292.75;3220.3)	2513.04 (2237.68;3228.99)	0.254	2585.51 (1669.57;3373.91)	2339.13 (1704.35;3211.59)	0.765

	Fenotipo		
	Classico N=32	Bulbare N=20	p-value
Endo EV, mediana (Q1-Q3)	220.29 (81.16;521.74)	315.94 (144.93;675.36)	0.2828
GLAST EV,mediana (Q1-Q3)	4.5 (3;5)	4 (3;8)	0.7034
Leuko EV, mediana (Q1-Q3)	568.12 (324.64;2237.68)	410.77 (211.59;916)	0.2385
PLT EV, mediana (Q1-Q3)	2295.65 (1669.57;3200)	2513.04 (1866.67;3228.99)	0.7565

	diff_ALSFRSR	diff_BMI	diff_FVC
	r (p-value)	r (p-value)	r (p-value)
Endo_ev	0.042 (0.7745)	0.287 (0.0555)	0.251 (0.1593)
Glast_ev	-0.103 (0.473)	-0.152 (0.3076)	-0.146 (0.4248)
Leuko_ev	0.168 (0.2486)	0.034 (0.8234)	0.266 (0.1351)
Plt_ev	0.011 (0.9382)	0.062 (0.6858)	0.181 (0.3147)
N	49	45	33

Figure 5. Correlation between all the EVs (Endo, Leuko, PLT and GLAST+ EVs) with the categorical variables (form of disease, mutation, progression, cognitive picture, phenotype) and not categorical clinical variables (delta ALSFRSR, delta BMI and delta FVC).

This project is funded by Italian Ministry of Education, University and Research program "Departments of Excellence 2018–2022"

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