Extracellular Vesicles From Participants With Long COVID Are Mannosylated and Bind to the *Galanthus nivalis* Agglutinin Resin in the Aethlon Hemopurifier

Miguel Pesqueira[1], Rosalia de Necochea Campion[1], Thomas Dalhuisen[2], Emily A. Fehrman[2], Jeffrey N. Martin[2], Timothy J. Henrich[2], Steven G. Deeks[2], Michael J. Peluso[2], Steven P. LaRosa[1]

1. Aethlon Medical Inc., San Diego, CA, USA
2. University of California, San Francisco, San Francisco, CA, US

Introduction

- Long COVID (LC) remains a substantial unmet medical need with no proven therapy[1]. Extracellular vesicles (EVs), nanoparticles released by cells and involved in cell-to-cell communication, have been implicated in the pathogenesis of this condition[2].
- The Aethlon Hemopurifier is an investigational device that combines plasma separation and affinity binding to a resin containing the plant lectin *Galanthus nivalis* agglutinin (GNA). GNA targets mannose on the surface of EVs and enveloped viruses (Fig. 1)[3].
- We hypothesized that the EVs present in Long COVID patients would be mannosylated, making them amenable to removal by the device.

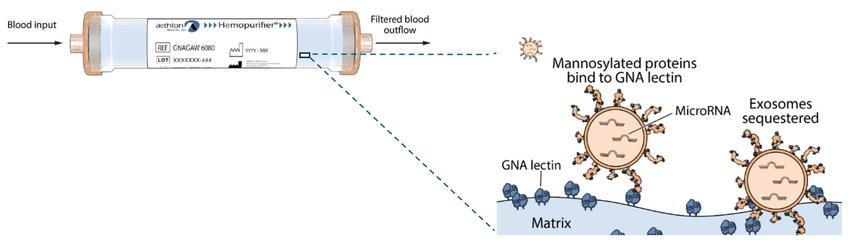


Figure 1. Extracellular Vesicle Sequestering by the Aethlon Hemopurifier GNA Affinity Resin.

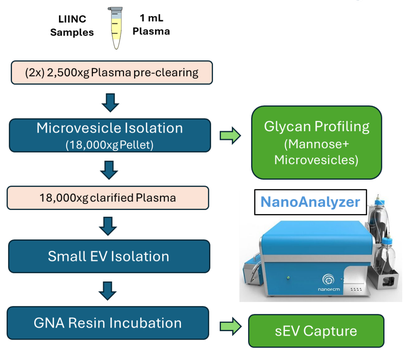
Participant Samples

- We studied plasma specimens from individuals enrolled in the UCSF Long-term Impact of Infection with Novel Coronavirus (LIINC) cohort approximately 90 days following COVID-19 symptom onset.
- Groups included: participants that had fully recovered from COVID-19 (No LC, n=15) and those who met the National Academies case definition for Long COVID with or without neurocognitive symptoms (NeuroLC, n=15; LC, n=15, respectively). For some analysis, LC and NeuroLC participants were grouped as 'All LC' (n=30) describing all symptomatic participants.

Methodology

- Microvesicles, >100 nm in diameter, were isolated by high-speed centrifugation and small EVs (sEV), 40-200 nm, were isolated by size exclusion chromatography.
- EV number was quantified using MemGlow, a phospholipid bilayer stain, and the NanoAnalyzer U30 instrument, a nanoparticle flow cytometry technology (NanoFCM Co., Ltd.).
- We incubated high-speed spun samples with soluble GNA-FITC and MemGlow to quantify mannose+ microvesicles.
- Purified sEV were mixed with GNA affinity resin and measured against a control to determine binding of GNA affinity resin to sEV.
- T-test analyses were used to compare differences between participant groups and Pearson's correlation coefficient (r) was used to compare the association between microvesicles and sEVs.

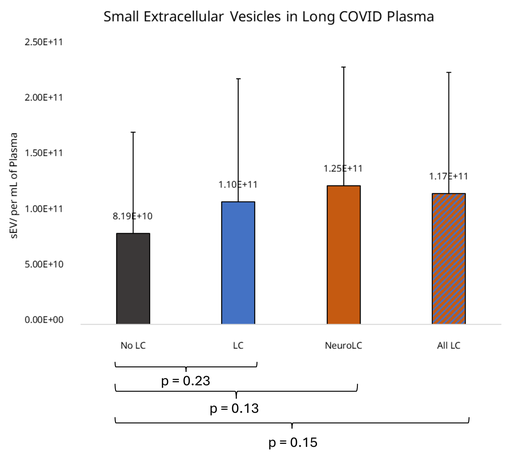
Overview of EV Isolation and Analysis Methods



Results

- EV counts were 30-40% higher in LC compared to full recovery (Fig. 2a-b; not statistically significant: p=0.15 and p=0.30, for sEV and microvesicles respectively).
- Among those with symptoms, a positive correlation between the quantities of small EVs and larger microvesicles was seen (Fig. 2c; r=0.61, p<0.001).
- A significantly higher quantity of mannose+ microvesicles was observed in participants with LC vs those fully recovered (Fig. 3a; 2.0E+08/mL vs. 8.8E+07/mL, p=0.04).
- We observed a significant correlation between microvesicles that bound GNA and removal of sEV by the affinity resin in symptomatic individuals but not in those that had recovered (Fig. 3b; r=0.34, p<0.05).

a.



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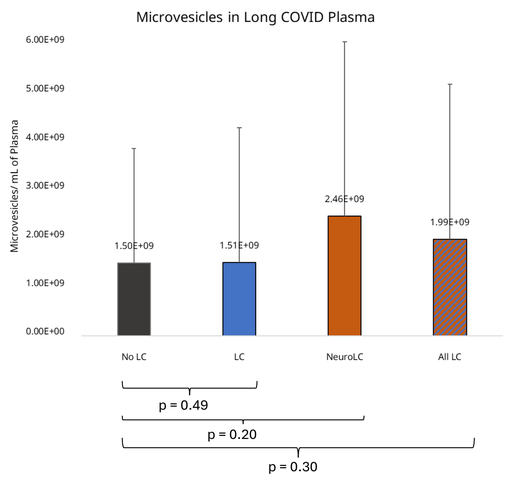

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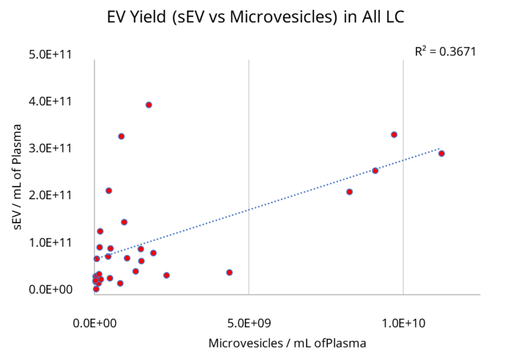
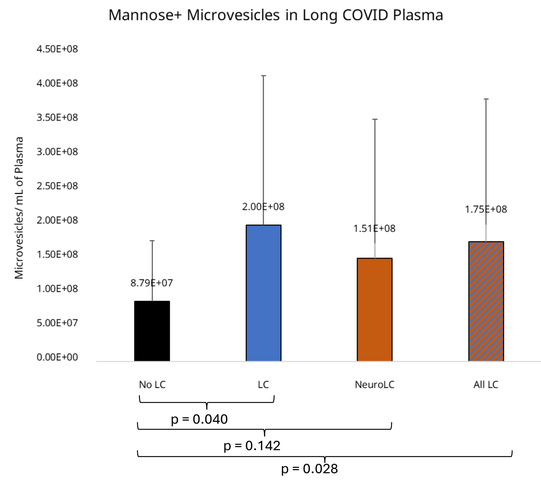


Figure 2. sEV and Microvesicle Yields in Long COVID Participant Plasma.

a.



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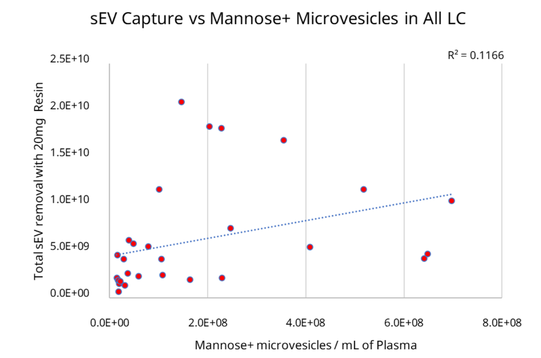

Figure 3. Mannose+ Microvesicles and Their Correlation with GNA Affinity Resin Removal of sEV in Long COVID Participant Samples.

Conclusions

- A significantly higher number of mannose+ microvesicles in participants with Long COVID versus those that recovered indicates a **unique glycosylation pattern in symptomatic individuals**.
- The significant correlation between mannose+ microvesicles and sEV removed by the GNA affinity resin suggests that **this EV glycosylation characteristic is present in both large and small vesicles**.
- The next step is to interrogate the cargo of EVs removed by the GNA affinity resin. Our findings support further evaluation of the Aethlon Hemopurifier as a therapeutic strategy in Long COVID.

References

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