Infection (2025) 53:2909–2912
https://doi.org/10.1007/s15010-025-02612-x

CORRESPONDENCE

Possible long COVID biomarker: identification of SARS-CoV-2 related protein(s) in Serum Extracellular Vesicles

Asghar Abbasi[1] · Ritin Sharma[2,3] · Nathaniel Hansen[2,3] · Patrick Pirrotte[2,3] · William W. Stringer[1]

Received: 20 June 2025 / Accepted: 16 July 2025 / Published online: 21 July 2025

Clinical trial registration number/other number: NCT05398692.

Clinical trial date of registration: 2022-05-10.

Emerging evidence suggests that SARS-CoV-2 RNA and viral antigens can persist in diverse tissues- (lung, brain, muscles, lymph nodes, and plasma)- for months to years after acute infection, and may be pathogenic for common long COVID symptoms [1, 2]. Extracellular vesicles (EVs)- nanosized vesicles (30–1000 nm) which facilitate intracellular communication of bioactive molecules such as proteins, lipids, nucleic acids, and metabolites [3]- have been found to harbor other viral RNA and proteins [4, 5], and may potentiate viral replication, immune activation and inflammation [6]. We propose that EVs with viral contents may serve as a potential biomarker in long COVID.

Blood samples were collected from 14 adults (aged ≥ 18 years) with a documented history of SARS-CoV-2 infection (confirmed via PCR or patient report) and persistent long COVID symptoms proposed by CDC and WHO (> 12 weeks since initial SARS-CoV-2 infection) including fatigue, dyspnea, exercise intolerance, or post-exertional malaise (PEM). The cohort was demographically and clinically diverse, including 43% women and 43% Hispanic/Latino participants. The majority (79%) were not hospitalized during their initial infection, and only one participant was unvaccinated at the time of study entry. Obesity was common (mean BMI 32.5 ± 8.4), and baseline physical activity levels were predominantly sedentary or limited to walking. The mean duration between initial SARS-CoV-2 infection and study enrollment was 17 ± 10 months. For further details about methods, see our previous publication [7]. Samples were obtained in response to acute incremental exercise (at rest and peak exercise), both before and after completion of the exercise training program. EVs were isolated from serum using precipitation kits (EQULTRA-20A-1, SBI Inc.) and analyzed by mass spectrometry-based proteomics. To assess whether any of the detected peptides were associated with SARS-CoV-2, the SARS-CoV-2 proteome (17 reviewed entries) was obtained from UniProt (UP000464024). EV samples were queried against a predicted SARS-CoV-2 spectral library in Spectronaut using default parameters (Fig. 1A).

Despite inter-individual variability, we detected 65 unique SARS-CoV-2 peptides mapping to the replicase polyprotein 1ab (Pp1ab; UniProt ID:P0DTD1, R1AB_SARS2), in 22 of the 56 serum EV samples (Fig. 1B). Sequence analysis (pBLAST) confirmed that these peptides were specific to SARS-CoV-2 and did not overlap with human proteins. Importantly, each subject exhibited one or more SARS-CoV-2 peptides in their EV cargo, suggesting the persistence of viral components over time (Fig. 1B). Pp1ab is encoded by the ORF1ab gene and plays a crucial role in viral RNA transcription and replication. To validate these findings, we applied a targeted mass spectrometry approach, using stable isotope-labelled synthetic (SIS) peptides (reference peptides) and high-resolution parallel reaction monitoring (PRM), to confirm the presence of Pp1ab-derived peptides in five randomly selected EV samples (Supplementary Fig. 1). We further verified the presence of a specific Pp1ab peptide ("GSLPINVIVFDGK") in serum EVs in 12 out of 14 long COVID subjects across multiple time points (20 out of 56 samples in total) (Fig. 1C). This peptide sequence (GSLPINVIVFDGK) corresponds specifically to non-structural

Patrick Pirrotte and William W. Stringer are co-senior authors.

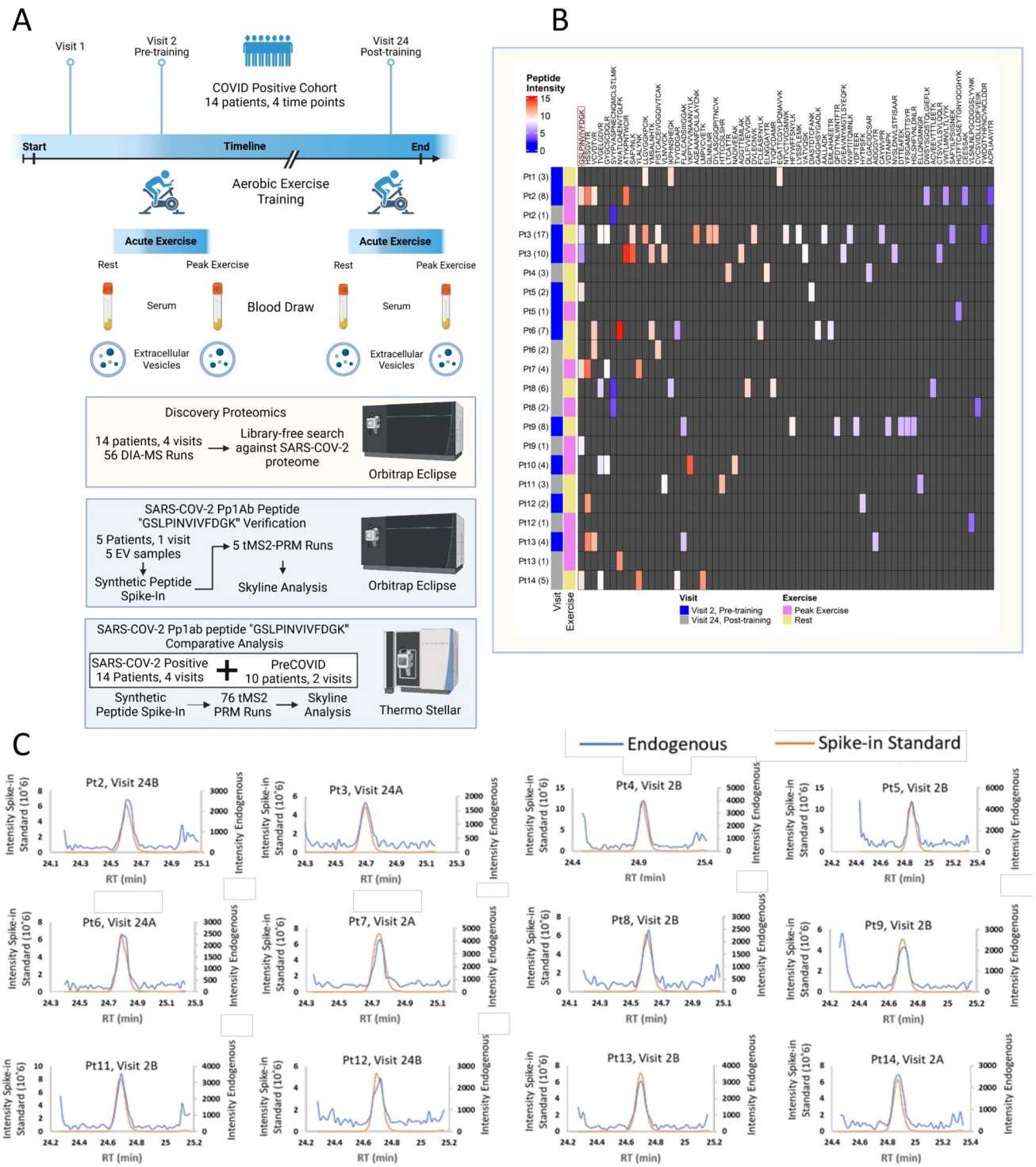
✉ Asghar Abbasi
asghar.abbasi@lundquist.org

William W. Stringer
stringer@ucla.edu

[1] The Lundquist Institute for Biomedical Innovation, Harbor-UCLA Medical Center, Torrance, CA, USA

[2] Early Detection and Prevention Division, Translational Genomics Research Institute, Phoenix, AZ, USA

[3] Integrated Mass Spectrometry Shared Resource, City of Hope Comprehensive Cancer Center, Duarte, CA, USA



protein 3 (nsp3) and is conserved across major SARS-CoV-2 variants, including Alpha, Delta, and Omicron, suggesting it is not variant-dependent.

To assess whether the peptides identified in long COVID EVs were also present in individuals without COVID-19 exposure (control group), we analyzed 20 serum EV samples collected prior to the COVID-19 pandemic (before 2019). These EV samples, obtained at rest and at peak incremental exercise from ex-smokers, showed no detectable viral peptide in any of the pre-pandemic specimens.

Overall, our targeted analysis confirmed the presence of the Pp1ab peptide in at least one sample in all long COVID subjects, while absent in controls. This finding will require confirmation in other long COVID studies with appropriate

◂**Fig. 1** Detection of SARS-CoV-2 peptides in serum EVs from individuals with long COVID. **A** Study design and analytical workflow. Blood samples were collected from individuals with long COVID in response to an acute exercise test (both at rest and peak exercise), before (visit 2) and after (visit 24) completing an exercise training program. Serum EVs were isolated from 56 samples, and proteins were analyzed using mass spectrometry to search for viral peptides. Samples were analyzed by data-independent acquisition mass spectrometry (DIA-MS) with a library-free search against the predicted SARS-CoV-2 proteome. Five EV samples with positive identification of the SARS-CoV-2 polyprotein 1ab (Pp1ab) were further validated using targeted MS2 with a spiked-in stable isotope-labeled synthetic (SIS) peptide standard ("GSLPINVIVFDGK") (Supplementary Fig. 1). Subsequently, all 56 EV samples were analyzed by targeted MS2 on the Thermo Stellar mass spectrometer for enhanced sensitivity using the same SIS peptide standard. For comparison, 20 control EV samples- collected prior to the COVID-19 pandemic- were processed and analyzed identically using the targeted MS2 workflow with spiked-in SIS peptides. **B** Heatmap showing EV samples from each long COVID patient with detected SARS-CoV-2 Pp1ab peptides. Color intensity (blue to red) reflects peptide abundance, and the numbers in parentheses indicate how many distinct Pp1ab peptides were found in each sample. The data table is available in Supplementary Table 1. In total, Pp1ab peptides were detected in 22 of the 56 EV samples, with each subject exhibited at least one peptide in their EV cargo. The peptide "GSLPINVIVFDGK," highlighted in red, was selected for further validation by targeted MS2 analysis. **C** Overlay of extracted ion chromatograms (EICs) illustrating the SARS-CoV-2 peptide "GSLPINVIVFDGK" detected in EV samples from long COVID patients (endogenous), alongside the matching synthetic reference peptide (spike-in-standard). The matching chromatographic patterns between the endogenous and synthetic peptides confirmed the presence of "GSLPINVIVFDGK" in 12 out of 14 patients with a positive signal. Full chromatogram profiles and related data are provided in Supplementary Table 2. *EVs* extracellular vesicles, *Pt* patient, *visit 2* pre-training, *visit 24* post-training

controls to establish the durability and sensitivity/specificity of this peptide biomarker. The detection of Pp1ab-laden EVs highlights the potential role of EVs in transporting viral material and suggests that these Pp1ab-laden EVs could serve as a potential biomarker for ongoing viral activity in the host, offering new avenues for diagnosis and therapeutic development in long COVID.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s15010-025-02612-x.

Acknowledgements The authors would like to express their gratitude to Melissa Martinez for her assistance with sample collection and handling in this study.

Author contributions A.Abbasi contributed to conceptualization, methodology, validation, investigation, resources, data curation, writing original draft, writing—review and editing, visualization, supervision, project administration and funding acquisition. R.Sharma was involved in methodology, validation, formal analysis, investigation, writing—review and editing and visualization. N.Hansen was involved in formal analysis, data curation, writing—review and editing and visualization of this manuscript. P.Pirrotte was involved in methodology, writing—review and editing, visualization, supervision of this manuscript. W.W.Stringer contributed to conceptualization, methodology, validation, investigation, data curation, writing original draft, writing—review and editing, visualization, supervision of the study and this manuscript, project administration and funding acquisition.

Funding This study was supported by the Pulmonary Education and Research Foundation (PERF) and the UCLA David Geffen School of Medicine (DGSoM)-Ventura County Community Foundation (VCCF) Long COVID 19 Research Award.

Data availability Data are available with appropriate requests to the corresponding author.

Declarations

Conflict of interest Asghar Abbasi is supported by TRDRP (28FT-0017), NIH (R43HL167289, 2R44HL167289-02), and the Johnny Carson Foundation. William Stringer receives research foundation support from the Pulmonary Education and Research Foundation and the UCLA David Geffen School of Medicine (DGSoM)-Ventura County Community Foundation (VCCF) Long COVID 19 Research Award. He reports consulting fees from Verona, Genetech, and Vyaire. He is the co-author of a clinical exercise testing physiology textbook for Lippincott.

References

1. Menezes SM, Jamoulle M, Carletto MP, Moens L, Meyts I, Maes P, et al. Blood transcriptomic analyses reveal persistent SARS-CoV-2 RNA and candidate biomarkers in post-COVID-19 condition. Lancet Microbe. 2024;5(8): 100849.
2. Peluso MJ, Swank ZN, Goldberg SA, Lu S, Dalhuisen T, Borberg E, et al. Plasma-based antigen persistence in the post-acute phase of SARS-CoV-2 infection. medRxiv. 2023. https://doi.org/10.1101/2023.10.24.23297114.
3. Buzas EI. The roles of extracellular vesicles in the immune system. Nat Rev Immunol. 2022. https://doi.org/10.1038/s41577-022-00763-8.
4. Vora A, Zhou W, Londono-Renteria B, Woodson M, Sherman MB, Colpitts TM, et al. Arthropod EVs mediate dengue virus transmission through interaction with a tetraspanin domain containing glycoprotein Tsp29Fb. Proc Natl Acad Sci U S A. 2018;115(28):E6604–13.
5. Tang Z, Lu Y, Dong JL, Wu W, Li J. The extracellular vesicles in HIV infection and progression: mechanisms, and theranostic implications. Front Bioeng Biotechnol. 2024;12:1376455.

6. O'Brien K, Breyne K, Ughetto S, Laurent LC, Breakefield XO. RNA delivery by extracellular vesicles in mammalian cells and its applications. Nat Rev Mol Cell Biol. 2020;21(10):585–606.
7. Abbasi A, Gattoni C, Iacovino M, Ferguson C, Tosolini J, Singh A, et al. A pilot study on the effects of exercise training on cardiorespiratory performance, quality of life, and immunologic variables in long COVID. J Clin Med. 2024;13(18):5590.