Exploring the Molecular Pathways of Long COVID with Post-Exertional Fatigue: A Multi-Omic Approach

Silvia Chafino[1,2], Maria Nevot[3], Cora Loste[4], Francisco Muñoz-López[3], Gemma Lladós[4], Cristina Lopez[4], José Ramón Santos[4], Bonaventura Clotet[3,4], Roger Paredes[2,3,4], Francesc Vidal[1,2,5], Joaquim Peraire[1,2,5], Lourdes Mateu[4], Anna Rull[1,2,5], **Marta Massanella2,3**.

[1]Infection and Immunity Research Group (INIM), Institut Investigació Sanitària Pere Virgili (IISPV), Hospital Universitari de Tarragona Joan XXIII (HJ23), Tarragona, Spain; [2]Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain; [3]IrsiCaixa, 08916 Badalona, Spain; [4]Fight Infections Foundation, Service of Infectious Diseases, Hospital Universitari Germans Trias i Pujol, Badalona, Spain; [5]Universitat Rovira i Virgili (URV), Tarragona, Spain.

938

mmassanella@irscaixa.es

BACKGROUND

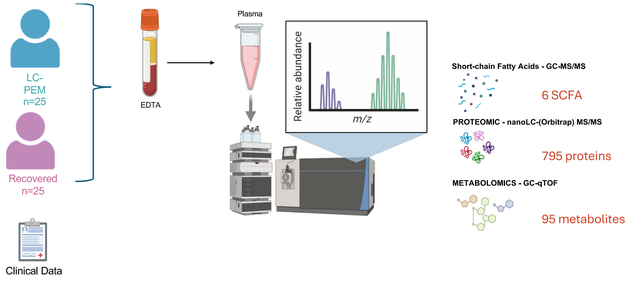
- Post-exertional malaise (PEM) is a disabling condition characterized by worsened symptoms after mental or physical exertion, commonly seen in Long-COVID (LC).
- While its underlying pathophysiology is unclear, potential mechanisms include oxidative stress, and metabolic derangements.

AIM

To conduct a multiomic analysis to identify changes in energy metabolism, immune function and cellular stress responses in patients with LC affected by PEM (LC-PEM).

METHODS

- **Study cohort:** We included 25 LC-PEM and **25 COVID-19 survivors** without persistent symptoms (Recovered) matched by sex, age and COVID-19 severity. We
- **Lipidomic, Metabolomic and proteomic analysis**



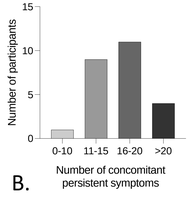
- **Statistical Analyses** included t-tests, partial-least square discriminant analysis (PLS-DA), Random Forest, Joint-Pathway analyses (KEGG database) and ROC Curve.

Table 1. Characteristics of the cohort

	Long-COVID with Post-exertional Malaise N=25	Recovered N=25
Female, n (%)	22 (88)	22 (88)
Age, years, median [IQR]	44 [36-54]	44 [36-53]
Days between sample collection and symptoms initiation, median [IQR]	252 [207-270]	230 [194–244]
Vaccinated at the moment of sampling, n (%)	0 (0)	0 (0)
Hospitalized, n (%)	6 (24)	7 (28)
Severe acute COVID-19, n (%)		
No oxygen	2 (8)	3 (13)
Low Flow oxygen	2 (8)	2 (8)
High Flow oxygen	2 (8)	2 (8)
Comorbidities, n (%)		
Arterial Hypertension	4 (16)	1 (4)
Dyslipidemia	7 (28)	6 (24)
Obesity	10 (40)	4 (16)
Allergy	12 (48)	4 (20)
Automimmune disease	3 (16)	1 (4)
HIV	0 (0)	1 (4)
Cefalea	2 (8)	2 (8)
Insomnia	4 (16)	1 (4)
Chronic Fatigue Syndrome	2 (8)	0 (0)
Fibromyalgia	2 (8)	0 (0)
Post-Viral fatigue syndrome	2 (8)	0 (0)
COVID-19 diagnostic, n (%)		
PCR/TAR	11 (44)	17 (68)
Serology	6 (44)	8 (32)
Clinical	8 (32)	0 (0)
Number of symptoms at sampling, median [IQR]	17 [14-19]	-
Range of symptoms	5-24	-

Significant alterations in energy metabolism, coagulation, and complement pathways in LC-PEM highlight the need for a multifaceted management approach targeting both metabolic and immune dysregulation.

RESULTS



> In addition to post-exertional malaise, participants commonly reported, Neurocognitive complaints (96%), dyspnea (92%), headache (84%), arthralgia (84%) & neurosensitive alterations (80%)

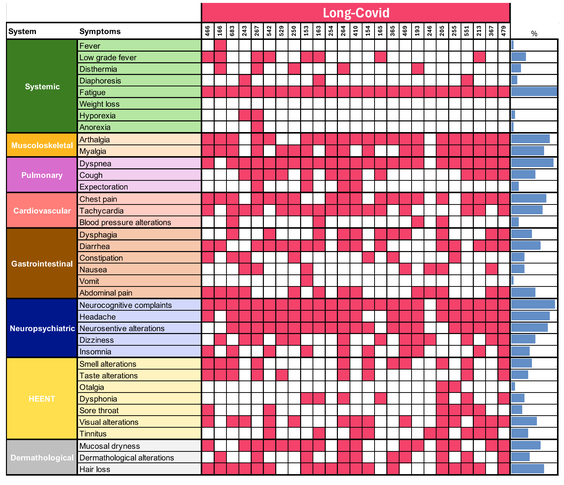


Figure 1. **Symptoms overview of LC-PEM.** Distribution of the number of symptomsa among participants (**A**) and heat map of the persistent symptoms (**B**)

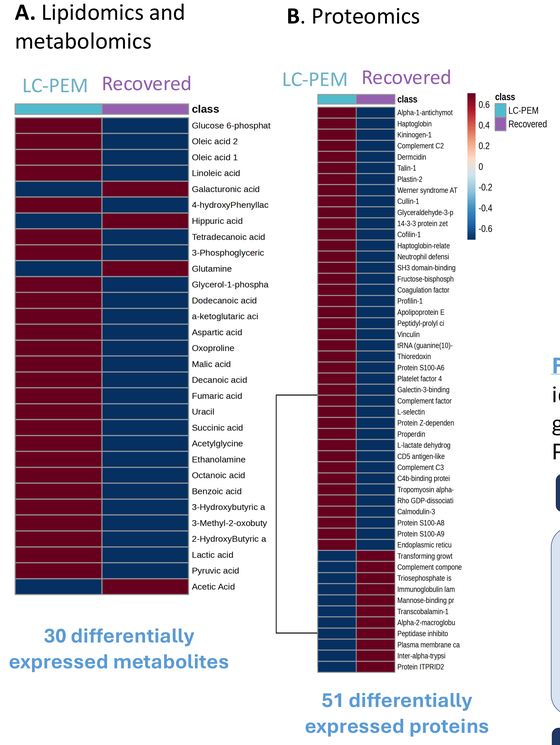


Figure 2. Metabolomics, lipidomics (A) and proteomics analysis between LC-PEM and recovered (B).

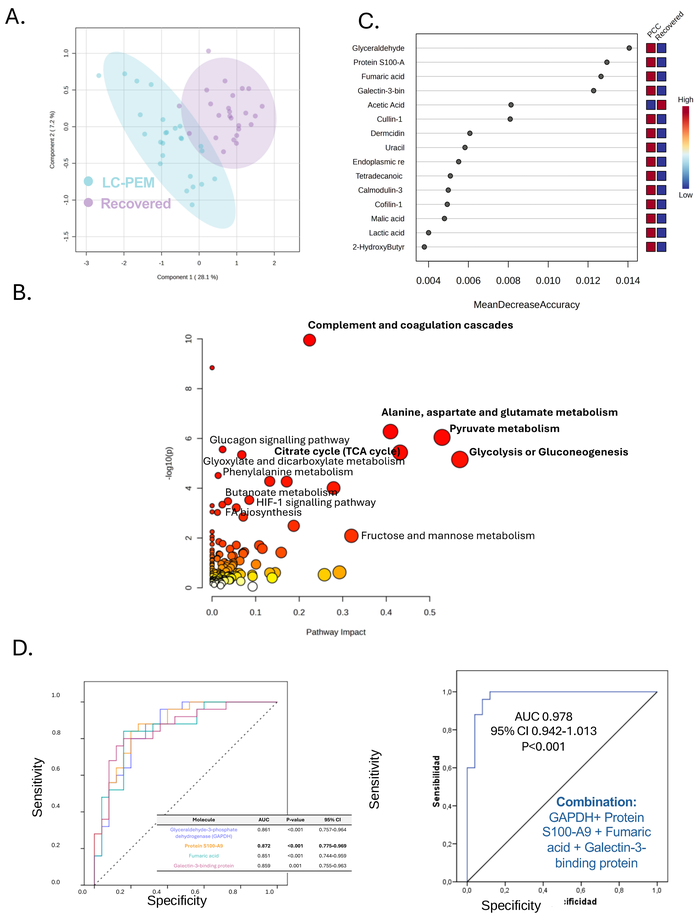


Figure 3. **Multi-omic analysis**. **A**. PLS-DA analysis of the 81 identified molecules shows distinct molecular profiles between the groups. **B**. Pathway analysis identifies 35 altered pathways in LC-PEM. **C**. Random forest and **D.** Roc curves of the best candidates

CONCLUSIONS

- Significant alterations in energy metabolism were observed with LC-PEM, including disruptions in the amino acid metabolism, TCA cycle, fatty acid biosynthesis and glycolysis.
- Alterations in coagulation and complement pathways were identified in LC-PEM, with 19 proteins exhibiting significant changes in expression.

ACKNOWLEDGEMENTS

This work was supported by CIBERINFEC groups (CB21/13/00063 and CB21/13/00020), Gilead grant (GLD21/00070). MM was supported by the was granted with RYC2020-028934-I/AEI/10.13039/501100011033 from Spanish Ministry of Science and Innovation and State Research Agency and the European Social Fund "investing in your future".