Characterization of Circulating Biomarkers Levels in Participants With Long Covid

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Ninon Marmont[1], Alejandro Abner Garcia Leon[1], Grace Kenny[1,2], Colette Gaillard[1], Eoghan de Barra[4], Eoin Feeney[1,2], Stefano Savinelli[1,2], Mary Horgan[6], Obada Yousif[5], Aoife G Cotter[1,3], Patrick WG Mallon[1, 2]

[1]Centre for Experimental Pathogen Host Research, University College Dublin, Dublin, Ireland, [2]St Vincent's University Hospital, Dublin, Ireland., [3]Mater Misericordiae University Hospital, Dublin, Ireland Beaumont Hospital, Beaumont, Dublin 9, Ireland, [4]Department of International Health and Tropical Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland, [5]Wexford General Hospital, Wexford, Ireland, [6]Department of Infectious Diseases, Cork University Hospital, Wilton, Cork, Ireland

BACKGROUND

- The pathogenesis of post-acute sequelae of COVID19 remains poorly understood, with a number of different clinical phenotypes reported (Kenny G *et al OFID* 2022)
- We aimed to characterize and identify host inflammatory patterns in people with Long COVID and their association with demographic factors, comorbidities, and symptoms.

METHODS

- Participants enrolled in the All Ireland Infectious Diseases (AIID) Cohort with PCR-confirmed COVID-19 diagnosed with Long COVID (symptoms ≥4 weeks post-acute COVID-19).
- We measured 57 biomarkers in plasma samples associated with: systemic inflammation, antiviral cytokine responses, endothelial dysfunction, tissue damage, coagulation, microbial translocation, innate immune activation, immune regulation and axonal injury. Luminex (Figure 1 A) and MSD assays were used for the analysis (Figure 1 B).
- We used principal component analysis and unsupervised hierarchical clustering to identify distinctive biological clusters. We used Kruskal-Wallis and Chi-Square tests to explore demographic and clinical parameter differences between clusters.

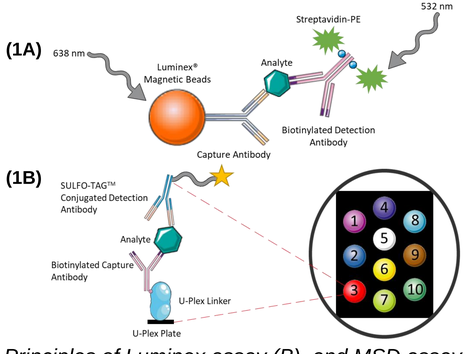


Fig 1: (A) Principles of Luminex assay (B), and MSD assay.

RESULTS

- From 364 participants (median [IQR] age 44 (35-53) years, BMI 27 (22-32), 74% female, 85% Caucasian), 6 distinct biological clusters were identified (Fig 2).
- Clusters 1, 2 and 3 (68,9%) displayed low levels of inflammatory biomarkers, lower BMI, younger age, and fewer comorbidities.

- We identified **six distinct biological patterns** among **long COVID** participants.
- **Three inflamed clusters** were characterized by **high levels** of biomarkers suggesting **tissue damage-coagulation**, **axonal injury** and **antiviral immune responses**.
- These inflamed clusters showed **significant associations** with **cardiovascular** and **respiratory symptoms** in fully **adjusted analysis.**

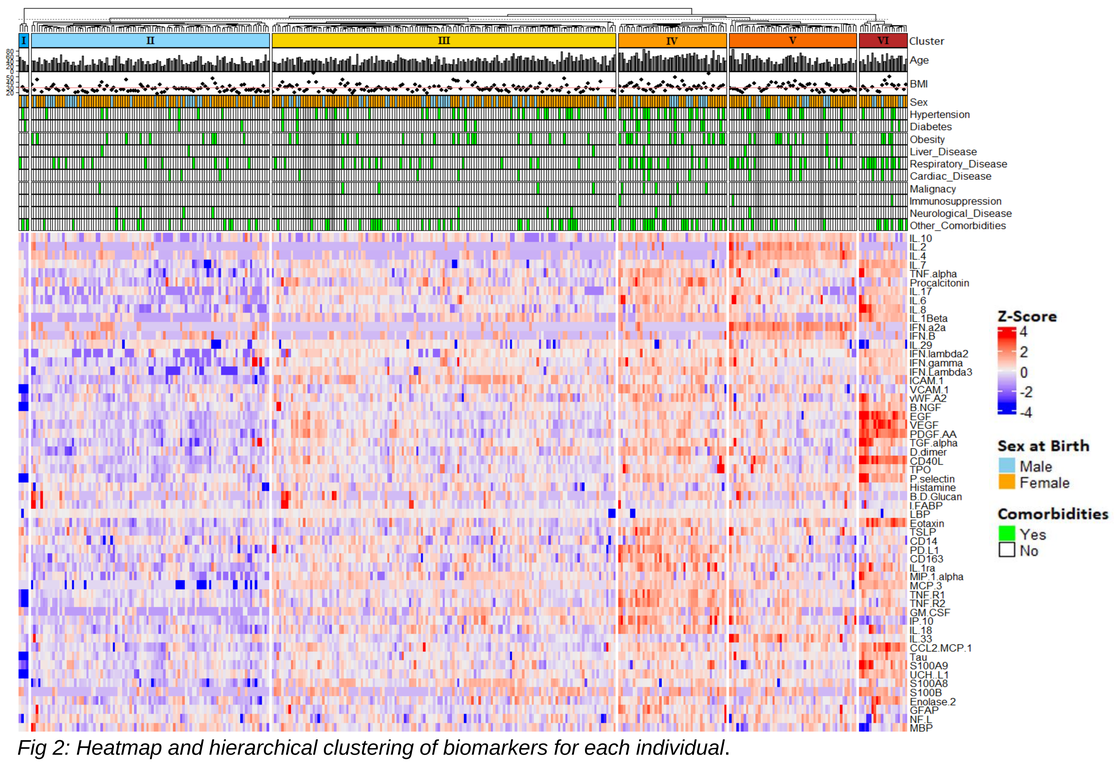


Fig 2: Heatmap and hierarchical clustering of biomarkers for each individual.

- Clusters 4, 5 and 6 were characterized by higher levels of inflammation.
- **Cluster 4** (12%) is characterized by higher innate immune activation markers, had the highest BMI, oldest age (median [IQR] age 57 (49-65) years), and higher prevalence of comorbidities such as hypertension and obesity, reflecting a biological phenotype suggesting metabolic syndrome.
- **Cluster 5** (14%) had higher anti-inflammatory (IL2, IL4, IL10) and antiviral biomarkers (IFN-α2a, IFN-B), suggesting a persistent immune response to infection, and had higher representation of younger age (median [IQR] age 46 (31-55) years).
- **Cluster 6** (5,5%), had higher levels of tissue damage biomarkers (VEGF, EGF, PDGF, TGF-α, β-NGF), coagulation (CD40L, P-selectin, TPO, vWF), and axonal injury (S100A9, S100B, Tau, ENO2, UCH-L1).

- In multivariate analysis, using the less inflamed clusters 1&2 as a reference category, and adjusting for demographic characteristics, we observed that prevalence of cardiac and respiratory symptoms of Long COVID were strongly associated with Cluster 6 (odds ratio of 5.6 (95% CI 1.1, 28.4) and 5.4 (95% CI 1.7, 17.7), respectively, Figure 3).

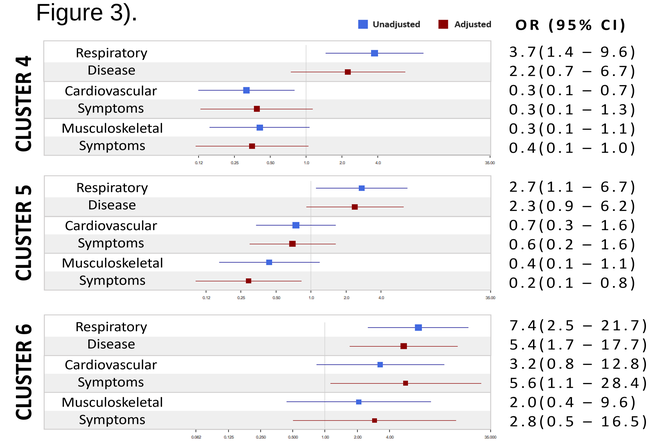


Fig 3: Multivariate model adjusted for age, sex at birth, ethnicity, BMI, smoking status, hypertension and diabetes.

CONCLUSIONS

- We identified **six distinct biological clusters** among long COVID participants, each with **unique clinical** and **biological characteristics**.
- Clusters with elevated levels of markers of **tissue damage**, **coagulation** and **axonal damage** (cluster 6), showed significant **associations** with **cardiac** and **respiratory symptoms** of Long COVID in adjusted analysis.
- These findings suggest **distinct underlying pathogenic** mechanisms in people with **long COVID**, highlighting the importance of recognizing these **biological differences** for potential **targeted treatments**.

ADDITIONAL KEY INFORMATION

The authors wish to thank all study participants and their families for their participation and support in the conduct of the All Ireland Infectious Diseases Cohort Study.

Author Contact Information : Ninon Marmont, Centre for Experimental Pathogen Host Research, University College Dublin, Dublin, Ireland, email: ninon.marmont@etu.uca.fr/ ninonmarmont@orange.fr, Phone: +33 6 35 11 45 08