Two Contributions to BELVIR 2025 — Transcriptomic Studies on Long COVID

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Summary by Marc Jamoulle

Two major studies were presented at the 13th annual BELVIR symposium, illustrating how blood transcriptomics, biomarker analysis, and advanced modeling approaches help shed light on the mechanisms of Long COVID and their clinical impact. These studies combine molecular data, functional indicators, imaging, and therapeutic variables to obtain an integrated view of viral persistence and its consequences on patients' overall health.

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Study 1 — Analysis of the Long COVID Biomarker Network

(Poster by Daniel Sanson, abstract below)

This study is based on two complementary approaches: supervised variable selection using the Boruta algorithm and a correlation network model. It shows that Long COVID is a syndrome characterized by an interwoven set of inflammatory, immune, platelet-related, and viral signals, revealing central markers such as IL-18, PTGS2, and HDAC5, as well as significant correlations with treatments.

Study 2 — Neutralizing Antibodies and Viral Persistence

(Oral presentation by Ms. Chenyu, abstract below)

This study demonstrates that Long COVID patients maintain a normal vaccine response, but that the broad neutralization observed is paradoxically associated with viral persistence, notably through ORF3a. It highlights a particular immune profile for the Delta variant, dominated by an atypical IgE response.

3. The Central Role of the COOP–WONCA Functional Indicator

The COOP–WONCA Charts were used as a global health assessment tool. The analyses show important correlations between COOP scores and biomarkers such as IL-18, PTGS2, and HDAC5, making it possible to link the transcriptomic signature to functional impact.

4. ORF1ab and Mpro: Markers of Viral Persistence and Therapeutic Keys

ORF1ab is a direct indicator of residual viral replication. Mpro, the main protease of SARS-CoV-2, is essential for viral replication. Detection of ORF1ab implies the presence of Mpro. Paxlovid, an Mpro inhibitor, is associated with clinical improvement and reduced inflammation.

5. Overall Clinical Summary

Long COVID appears as an objectifiable syndrome characterized by viral persistence, chronic immune activation, and impaired functional health. The COOP–WONCA tools are essential for linking molecular biology to clinical reality, while the identified biomarkers open the path toward personalized Long COVID medicine.

POSTER 10

Abstract 10 | Desentangling the Long Covid biomarker network: Insights into viral persistence and therapeutic response using random forest feature selection

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Long COVID, also referred to as post-acute sequelae of SARS-CoV-2 infection (PASC), is a complex multisystem syndrome characterized by persistent symptoms across diverse biological systems. Accumulating evidence indicates SARS-CoV-2 viral persistence and subsequent inflammation, coagulation defects, auto-antibody development, and tissue damage as root causes. Our groups and others have demonstrated viral RNA and protein persistence (SIMOA) up to 2 years after acute COVID. To identify biomarkers predictive of clinical outcome, Boruta, a supervised feature selection algorithm based on random forest classification, was utilized to objectively identify demographic, clinical (patient-reported COOP score, clinician-reported Long COVID grade), and molecular (nCounter digital transcriptomics) predictors from 78 patients with long-term follow-up. In addition to the variables selected by Boruta, age, sex, vaccination status, COVID episodes, viral RNA load (VL), and SARS-CoV-2 ORF1ab (protease target of Paxlovid) transcripts were added based on prior biological relevance. A Gaussian graphical model was then established, applying a correlation-based approach to visualize and examine the interdependencies among selected variables. The resulting correlation network revealed prominent clusters and interconnected modules involving immunological markers (e.g., *IL18* with COOP Feeling, $r = 0.32$, $p = 0.0075$; *HDAC5* with COOP Change, $r = 0.39$, $p = 0.00094$), clinical scores and patient-reported outcomes, and therapeutic interventions (e.g., antiplatelet therapy with Long COVID grade, $r = -0.43$, $p = 0.00044$; antiviral therapy (Paxlovid) with Long COVID grade, $r = -0.27$, $p = 0.026$). The variable Delta COOP, representing temporal changes in patient-reported general health status, exhibited significant correlations with specific RNAs including *PTGS2* ($r = 0.41$, $p = 0.0081$), *DDX50* ($r = -0.31$, $p = 0.045$), and *NCF2* ($r = 0.41$, $p = 0.0083$), indicating its relevance in assessing disease progression, identifying central nodes with high connectivity. VL was inversely correlated with *PTGS2* ($r = -0.36$, $p = 0.0012$), and positively correlated with *HDAC5* ($r = 0.31$, $p = 0.0061$). Of note, both are promising therapeutic targets since *PTGS2*, which encodes COX-2, is inhibited by well-known anti-inflammatory drugs such as aspirin and ibuprofen, while the *HDAC5* gene can be targeted by FDA-approved histone deacetylase inhibitors including Vorinostat, Panobinostat, and Belinostat. Moreover, Paxlovid use also showed a negative correlation with *IL18* ($r = -0.25$, $p = 0.028$). The analysis underscored complex interplay between immune mediators, platelet-related markers (*IL12A/HDAC5*), and symptom domains, further identifying time after acute COVID as a key factor of *TLR7* ($r = 0.42$, $p = 0.0002$), Long COVID grade ($r = 0.43$, $p = 0.0003$) and viral load ($r = -0.39$, $p = 0.0006$).
In conclusion, combining supervised Boruta selection and network correlation analysis provides a comprehensive framework for characterizing the intricate biology of Long COVID and prioritizing candidate viral (VL) and/or immune biomarkers (IL18) or therapeutic targets (PTGS2/HDAC5).

SHORT TALK

Abstract 12 | Broadly neutralizing antibodies to SARS-CoV-2 in Long COVID are associated with viral persistence and clinical outcome

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Long COVID (also known as post-acute sequelae of SARS-CoV-2, PASC) is a chronic condition in which symptoms persist months or years after the acute infection. Neutralizing antibody responses induced by infection and/or vaccination are a surrogate marker for protection against COVID-19. However, their potential role in Long COVID patients is currently unclear. Herein, we performed pseudoneutralization assays (MSD) of 10 different SARS-CoV-2 variants and whole blood digital transcriptomics (nCounter, Nanostring) in a real-world, perspective Long Covid cohort (n=49 paired samples), examining possible correlations with clinical (brain imaging (SPECT-CT), severity, COOP chart scores) and demographic data. Across all variants, the number of vaccine doses was significantly correlated with broad neutralization activity ($P<0.001$, $R>0.4$ for WT, Alpha, Beta, Gamma, Delta), indicating vaccine response is not defective in Long COVID patients. Moreover, broad neutralization activity was positively correlated with SARS-CoV-2 ORF3a RNA ($P= 0.0085$, $R = 0.37$), consistent with previous findings of viral persistence in Long COVID, while inflammatory chemokines CCL2 and CXCL10 ($P=0.016$, $R=-0.346$) were negatively correlated with broad neutralizing antibodies. Interestingly, for the Delta variant, a unique transcriptomic profile was observed compared to all other variants, with negative correlations to IgE heavy chain RNA (*IGHE* $P=0.00085$, $R=-0.37$) and cell cycle-related transcript *CDC20* ($P=0.00085$, $R=-0.37$). This points at an IgE-dominated humoral response to the Delta variant, which may help explain both its higher virulence and immune escape from neutralizing antibodies. Although middle-aged women are more frequently affected by Long COVID, age and sex were not significantly associated with neutralizing antibodies to any variant. From a clinical standpoint, broadly neutralizating antibodies were unexpectedly associated with worse clinical outcome, quantified by COOP score changes from baseline (higher values indicate worsening, $P= 0.0048$, $p = 0.42$). In conclusion, our data suggest the presence of a viral reservoir in Long COVID that continuously stimulates neutralizing antibody production, which is however associated with worse clinical outcome. Humoral immune response to the Delta variant is skewed to IgE dominance in Long COVID patients, in keeping with its higher virulence.