

# **A multi-omics recovery factor predicts long COVID in the IMPACC study**

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## **ABSTRACT**

**Background.** Following SARS-CoV-2 infection, ~10-35% of COVID-19 patients experience long COVID (LC), in which debilitating symptoms persist for at least three months. Elucidating biologic underpinnings of LC could identify therapeutic opportunities.

**Methods.** We utilized machine learning methods on biologic analytes provided over 12-months after hospital discharge from >500 COVID-19 patients in the IMPACC cohort to identify a multi-omics “recovery factor”, trained on patient-reported physical function survey scores. Immune profiling data included PBMC transcriptomics, serum O-link and plasma proteomics, plasma metabolomics, and blood CyTOF protein levels. Recovery factor scores were tested for association with LC, disease severity, clinical parameters, and immune subset frequencies. Enrichment analyses identified biologic pathways associated with recovery factor scores.

**Results.** LC participants had lower recovery factor scores compared to recovered participants. Recovery factor scores predicted LC as early as hospital admission, irrespective of acute COVID-19 severity. Biologic characterization revealed increased inflammatory mediators, elevated signatures of heme metabolism, and decreased androgenic steroids as predictive and ongoing biomarkers of LC. Lower recovery factor scores were associated with reduced lymphocyte and increased myeloid cell frequencies. The observed signatures are consistent with persistent inflammation driving anemia and stress erythropoiesis as major biologic underpinnings of LC.

**Conclusion.** The multi-omics recovery factor identifies patients at risk of LC early after SARS-CoV-2 infection and reveals LC biomarkers and potential treatment targets.

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## INTRODUCTION

Long COVID (LC) has become a pressing public health concern, affecting ~10-35% of surviving individuals infected with SARS-CoV-2, or 15-20 million individuals in the United States (US) and >60 million worldwide (1,2). In July 2024, the National Academies of Sciences, Engineering, and Medicine (NASEM) released an updated definition of LC, characterizing it as a chronic condition arising after SARS-CoV-2 infection that persists for at least 3 months, irrespective of acute disease severity (2). LC can encompass a wide range of physical and cognitive symptoms, and can lead to new or worsening neurological, psychiatric, cardiovascular, pulmonary, endocrine, and gastrointestinal conditions, among others (1–12).

Several studies have identified demographic and clinical risk factors for LC (13–15), including older age (16–18), female sex (16–21), and longer hospital stays (18,22). Higher viral loads (18,19) and lower anti-SARS-CoV-2 antibody titers (18,23,24) during the acute infection phase have also been associated with LC development. Several non-mutually exclusive hypotheses may explain the etiology of LC, including persistent viral infection (25,26), chronic inflammation (26–29), latent herpesvirus reactivation (26,30,31), immune dysregulation (29,32,33), complement dysregulation (34), and autoimmunity (35,36). Despite substantial efforts, no consensus exists on the mechanisms of LC pathogenesis, and validation of the molecular findings across cohorts has been challenging. Additionally, most existing studies rely on measurements from a single or limited number of assays and are confined to restricted sampling time points during the acute or convalescent phases of the disease. Accordingly, a large-scale multi-omics longitudinal study spanning both acute infection and convalescent disease phases could help elucidate the molecular mechanisms underlying LC.



The Immunophenotyping Assessment in a COVID-19 Cohort (IMPACC) study (37) offers a unique opportunity to investigate the temporal dynamics of multi-omics immune profiles during the acute and convalescent COVID-19 infection phases in a clinically well-characterized cohort of hospitalized patients from across the US. Data from the IMPACC study can be leveraged to identify molecular correlates of post-acute symptom development or resolution for one year after hospital discharge. This cohort has previously been studied to characterize multi-omics determinants of acute COVID-19 severity and mortality (38–40). Another study of the IMPACC cohort identified LC participants who experienced patient-reported outcome deficits up to 12 months after COVID-19 hospital discharge (18). Clinical characteristics such as female sex or a higher respiratory SARS-CoV-2 viral burden with lower antibody titers against the SARS-CoV-2 Spike protein during acute disease were associated with persistent deficits after hospital discharge. B cell lymphopenia and elevated fibroblast growth factor 21 (FGF21) during the acute phase were also characteristics of participants who developed LC (18). However, longitudinal immune profiles of IMPACC participants experiencing LC during the convalescent phase have not yet been compared to those of IMPACC participants who experienced minimal deficits during convalescence, an analysis that could reveal LC biomarkers and uncover biological processes underlying the disease.

In the current work, we applied supervised multi-omics integration methods to develop interpretable models that differentiate participants with LC from recovered individuals based on their longitudinal immunophenotyping profiles during the convalescent disease phase. We identified key biological programs and biomarkers driving LC classification. Our findings highlight persistent inflammation, elevated heme metabolism associated with anemia, and reduced plasma concentrations of androgenic steroids as characterizing features of LC, independent of acute disease severity or SARS-CoV-2 vaccination status post hospital discharge. Notably, these molecular profiles were already detectable during acute disease, suggesting their potential value

as early predictive biomarkers for identifying patients at risk of developing LC. Additionally, despite a general lack of consensus about the definition of LC or consistency in the timing of sampling across different studies, we validated dysregulation of the heme metabolism signature in two independent LC cohorts that included non-hospitalized COVID-19 patients. These findings provide valuable insights into the molecular underpinnings of LC and offer a foundation for future research aimed at improving diagnostics and developing targeted interventions.

## RESULTS

### Longitudinal multi-omics profiling of long COVID

The IMPACC study included 1,164 participants admitted to 20 US hospitals for COVID-19 infection between May 2020 and March 2021 (37). Clinical data collection and immunophenotyping were performed longitudinally during the acute disease phase within 72h of hospital admission and 4, 7, 14, 21 and 28 days after hospital admission (Visits 1-6, respectively). Surviving participants were contacted 3, 6, 9, and 12 months after hospital discharge (Visits 7-10, respectively) to complete patient-reported outcome and symptom surveys during the convalescent phase and to provide biosamples for immunophenotyping assays. Of the 702 participants who could be reached by the study team after discharge, 513 were included in the IMPACC Convalescent cohort (Figure S1). These participants were selected because they survived at least 28 days of hospitalization, completed at least one patient-reported outcome survey, and provided at least one biosample during the convalescent period (Figure 1A and Table S1)(18). IMPACC core labs performed immunophenotyping both in the acute and convalescent phases, including measurements of inflammatory mediators in blood serum via Olink (SO), global blood plasma metabolomics (PMG), global and targeted blood plasma proteomics (PPG and PPT), peripheral blood mononuclear cell (PBMC) transcriptomics (PGX), whole blood cell frequencies measured by mass cytometry by time of flight (CyTOF), and CyTOF mean marker signal intensity measurements (BCT).

LC status was defined in this cohort according to the participant's response to post discharge surveys that captured symptoms and Patient-Reported Outcome (PRO) measures evaluating general health and deficits in specific domains. Participants who responded to at least one set of post discharge surveys were assigned to PRO clusters according to latent class modeling and clustering using standardized scores of the PRO survey measures (18) (PRO survey score details

can be found in the Supplemental Methods). PRO clusters were classified as participant clusters with no or minimal deficits (MIN), or with deficits attributed to LC in several domains: physical predominant (PHY), mental/cognitive predominant (COG), and multi/pan domain (MLT) (18) (Figure 1B).

In this study, we utilized multi-omics immunophenotyping profiles from participant biosamples obtained during the convalescent disease phase to develop interpretable models for predicting LC and exploring the underlying molecular mechanisms. To assess model performance, we split the Convalescent cohort into an 80% Train and 20% Test cohort, maintaining the proportions of participants in each PRO cluster (Figure 1C), with no noticeable imbalance in other clinical characteristics or biosample availability between the cohorts (Figure S2). We then used Signature-based multiPle-omics intEgration via lAtent factoRs (SPEAR) (41), a supervised Bayesian factor model for the identification of multi-omics features, to integrate the high dimensional data and construct multi-omics predictive factors from immune profiles obtained during the convalescent phase in the Train cohort. We assessed their predictive performance by repeated cross-validation on the Train cohort and validated the performance of the selected model on the Test cohort (Figure 1, D and E). To identify immune programs captured in the predictive factors, we conducted in depth analyses of enriched biological pathways and analytes identified as highly relevant for the model's predictive performance and performed associations with assay data not included in model training, such as blood CyTOF cell frequencies.

### **Multi-omics factors are predictive of long COVID**

We focused on predicting LC in the Convalescent cohort using multi-omics immune profiling data collected during the convalescent phase. We constructed several SPEAR models to generate supervised factors. These models used either PRO survey scores from patient-reported outcomes measurement information system (PROMIS) surveys (SPEAR Physical, SPEAR Cognitive,

SPEAR Mental, SPEAR Impact, SPEAR Dyspnea) or the binary LC labels assigned to each participant (SPEAR LC) as response variables (Figure S3A). We trained models on these different response variables since binary LC labels (presence or absence of LC) per participant could omit valuable information captured by numeric PRO survey scores at each visit. Note that PRO survey scores were available for participants in all LC clinical PRO clusters, so each individual SPEAR model (e.g. SPEAR Physical) was trained using data from participants in all four PRO clusters (MIN, PHY, COG, MLT) (18). The SPEAR Physical model performed best among all models trained on PRO survey scores (Figure S3B), and outperformed the model trained on binary LC labels (Figure 2A). Additionally, all SPEAR models outperformed equivalent models trained on unsupervised multi-omics factors, obtained with the Multi-Omics Factor Analysis (MOFA) framework (42), which do not consider a response variable during the factor construction step (Figure 2A, Figure S3B). The SPEAR Physical model achieved an AUROC of 0.69 for predicting LC presence or absence in the Test cohort (Figure 2B). The SPEAR Physical Factor, learned by the SPEAR physical model, was significantly associated with LC in the Test cohort after correcting for sex and age ( $p=0.00098$ , effect size 0.44), two variables previously associated with LC in our cohort (18) (Figure 2C). Sparse lasso regression models to reconstruct SPEAR Physical factor scores utilizing all analytes included in the model or analytes from individual assays showed that the model which included all analytes best reconstructed factor scores, indicating that factor scores captured contributions from multiple omics (Figure S3C). The SPEAR Physical Factor scores were significantly higher for participants in the MIN group compared to the LC group, so we termed this factor the “recovery factor” (Figure 2C, Figure S4, Figure S5). Recovery factor scores were significantly associated with PRO clusters ( $p=0.0009$ ); however, they showed a differential ability to identify individual LC deficit domains, with significant differences between MIN vs COG ( $p=0.0042$ , effect size 0.61) and MIN vs MLT ( $p=0.0018$ , effect size 0.60) PRO clusters, but not MIN vs PHY clusters ( $p=0.28$ , effect size 0.47, Figure 2D, Figure S5B). Taken together, the recovery factor is a multi-omics model comprised of biologic analyte levels during

the convalescent phase of COVID-19 that distinguishes MIN from LC over 12 months post-hospital discharge in the IMPACC cohort.

### **Functional characterization of the recovery factor**

To characterize the biologic processes underlying the recovery factor, we performed gene set enrichment analysis (GSEA) for each of the multi-omics assays based on the SPEAR model's internal ranking of the relative importance of each feature for predicting the PRO PROMIS Physical score. The Hallmark Heme Metabolism transcriptomic pathway was negatively associated with the recovery factor, indicating upregulation in LC participants, whereas the androgenic steroids metabolite set was positively associated with the recovery factor, indicating downregulation in LC participants (Figure 3A). Evaluated individually, several leading edge analytes in the Hallmark Heme Metabolism gene set and androgenic steroids Subpathway metabolite set showed significant associations with LC status (Figure S6, A and B).

SPEAR performs internal significance testing to determine the importance of each analyte in predicting the response variable. The SPEAR Physical model identified 26 analytes across four assays as significant in the recovery factor (SPEAR Bayesian posterior selection probability  $\geq 0.95$ ), and we performed associations of these features with LC status in the test cohort, adjusting for age and sex (Figure 3B, Figure S6E). Nine of these 26 analytes were from the serum Olink assay. Of these, DNER, a non-canonical Notch ligand implicated in promoting tumor growth, metastasis, and wound healing (43,44) was significantly reduced in LC participants, consistent with a prior study of plasma proteins in LC (28). The remaining serum Olink analytes were negatively associated with the recovery factor. These included proteins and cytokines associated with chronic inflammatory conditions (45–50), particularly endothelial/vascular inflammation (FGF23, FGF21, CXCL9, TNFRSF11B and TNFRSF9 (CD137)), as well as inflammation-associated myeloid regulators (51–53) (MMP10 and CSF1). Elevated IL10RB levels have been

associated with worse outcomes in acute COVID-19 infection (54), consistent with elevation under inflammatory conditions. LRG1, a protein elevated in LC participants, is induced by IL-6 and other inflammatory cytokines and has been implicated in angiopathic activity (55–57). Phenylacetylglutamate and phenylacetylglutamine are gut microbiota-derived metabolites associated with vascular inflammation and thrombosis (58). Finally, the *OSBP2* transcript, which encodes an oxysterol binding protein (59), was a leading edge gene in the Hallmark Heme Metabolism gene set elevated in LC participants.

Several metabolites from the androgenic steroids pathway were represented in the 26 significant analytes and were positively associated with the recovery factor, indicating higher levels correlate with better physical function. When these metabolites were tested individually for association with LC status, five (DHEA-S, epiandrosterone sulfate, androsterone sulfate, 5alpha-androstan-3beta,17beta-diol monosulfate (2), 5alpha-androstan-3beta,17alpha-diol disulfate) were significantly lower in LC participants, adjusting for age and sex (Figure 3B). Androgens can suppress inflammation (60), suggesting that higher levels of androgenic steroids in MIN participants could reflect better control of chronic inflammation. These findings are consistent with reports showing lower sex hormone levels in LC (31). Five metabolites related to pregnenolone were also represented in the significant SPEAR analytes (Figure 3B). Pregnenolone is synthesized from cholesterol as the first step of the steroid hormone biosynthesis pathway and is known to have potent effects as an inhibitor of inflammation (61) and as a neurosteroid (62). Altogether, these findings are consistent with a prominent role for persistent inflammation in LC with dysregulation of key analytes that may contribute to LC symptoms, including those that drive angiopathy, reduce wound healing, and alter heme metabolism.

The feature sets from heme metabolism and androgenic steroids identified by GSEA combined with the significant SPEAR analytes represent 73 unique features that potentially condense the

predictive power of the recovery factor into a smaller feature set. To test this hypothesis, we calculated the geometric mean of the 43 leading edge heme metabolism and 12 androgenic steroid features, as well as the 26 significant SPEAR analytes. All three geometric mean scores were significantly associated with LC in the test cohort (Figure 3C). Furthermore, the combined score, including analytes from all three feature sets, discriminates MIN and LC participants with even greater significance (Figure 3C). Thus, while the recovery factor consists of weighted contributions from 6,807 features, we have identified a smaller set of 73 unique features that discriminates participants according to LC status in the convalescent period.

Consistent with our finding, two separate cohorts have reported upregulation of the Hallmark Heme Metabolism pathway in individuals with LC. In Hanson et al. (29), hospitalized and non-hospitalized participants with persisting symptoms 1-3 months after acute SARS-CoV-2 infection had higher Hallmark heme metabolism signatures than participants without persisting symptoms. In Karisola et al. (63), which included exclusively non-hospitalized COVID-19 patients, males with persisting symptoms three months after acute SARS-CoV-2 infection had higher Hallmark heme metabolism signatures compared to males without persisting symptoms. To determine whether the same heme metabolism-related genes were dysregulated in LC participants in the IMPACC and external cohorts, we used the leading edge genes from the significant Hallmark Heme Metabolism pathway in our GSEA results (Figure S6A) and calculated the geometric mean scores in whole blood transcriptomics profiles from Hanson et al. (29) and PBMC transcriptomics profiles from Karisola et al. (63). These heme metabolism leading edge genes significantly differentiated participants with persistent symptoms from those with resolved symptoms in both cohorts, including both sexes (Figure S6, C and D). The generalization of elevated expression of this heme metabolism gene set in non-hospitalized and hospitalized COVID-19 patients who experience LC in three independent and varied cohorts underscores its centrality for LC pathology.



Prior studies have identified altered leukocyte frequencies as a feature of LC (18,26,29,31,33). To determine whether similar cellular changes were associated with the recovery factor, we analyzed whole blood CyTOF cell frequencies for 15 parent and 46 child immune cell types in our cohort during convalescence (Figure 3D). Several cell subsets were significantly associated with the recovery factor. B cells and CD161<sup>+</sup> MAIT cells were positively associated with the recovery factor. In contrast, polymorphonuclear leukocytes (PMN) and monocytes, specifically the CD14<sup>+</sup>CD16<sup>-</sup> classical monocyte subset, were negatively associated with the recovery factor. Together, these findings suggest that elevated monocytes and neutrophils, along with decreased B cells, are associated with prolonged inflammation during LC. These findings are consistent with a previous report that monocytes are elevated in males with LC (31). The decrease in MAIT cells could be another effect of sustained inflammation as reduced circulation of MAIT cells has been associated with chronic HIV (64) and hepatitis C (65) viral infections.

#### **The recovery factor is associated with clinical characteristics and multiple patient reported outcomes in the convalescent period**

We next evaluated whether the recovery factor was associated with clinical features and additional clinical outcomes. We tested the association of recovery factor scores with clinical features at hospital admission (Visit 1), including demographics, comorbidities, complications, and baseline lab measurements (Figure 4A). Several demographic and clinical measures were significantly associated with recovery factor scores, including age, sex, length of hospital stay and the Sequential Organ Failure Assessment (SOFA) score. Notably, anemia at hospital discharge was negatively associated with the recovery factor, whereas hemoglobin and hematocrit baseline measurements showed a significant positive association (Figure 4A). We additionally conducted association testing with Patient Reported Outcome (PRO) scores from surveys conducted at the same visit at which the recovery score was assessed in participants across the convalescent period, correcting for age and sex. The recovery factor score was significantly associated in the

test cohort with the PROMIS Physical score, on which the model was trained (Figure 4B), and the EQ-5D-5L score, both of which contained questions assessing physical function (Figure 4B). The recovery factor also correlated with PROMIS Mental and Psychosocial Impact scores, although these associations were not significant after p-value correction (Figure 4B). We also tested whether recovery factor scores associated with reported clinical symptoms in the 7 days prior to each visit but found no significant association (Figure 4C).

There is a lack of consensus about whether LC is associated with the severity of acute disease. A previous analysis of clinical features from the IMPACC cohort showed no association between acute infection severity, as assessed by clinical trajectory groups, and LC development (18). However, other studies have found an association (6). Thus, we sought to determine whether acute disease severity contributed to the association between recovery factor scores and LC status in our cohort. Clinical severity in the IMPACC cohort during the acute phase was defined by unsupervised clustering of respiratory ordinal score over time, taking discharge status and limitations into account, with trajectory group 1 (TG1) representing the mildest and TG4 the most severe disease course among participants who survived for at least 28 days post hospitalization (66). After correcting for acute phase trajectory group assignment, recovery factor scores remain significantly associated with LC at the first three convalescent time points (Figure 4D), indicating that acute clinical severity does not contribute to the association between participant recovery factor scores in the convalescent disease phase and LC status.

### **Sex impacts recovery factor scores**

LC occurs more frequently in females than males, despite a higher percentage of males with severe acute COVID-19 disease (21,67). In the IMPACC Convalescent cohort, nearly half of the female participants presented with long term deficits compared to only ~30% of male participants (Figure S7A). Assignment to clinical LC subtypes was not influenced by sex, with similar

proportions and numbers of male and female LC participants assigned to COG, PHY and MLT PRO clusters (Figure S7A). However, consistent with the known influence of sex on LC status, sex was a statistically significant covariate in the recovery factor association with LC status from Figure 2C ( $p=3.6e-7$ ) and with PRO clusters from Figure 2D (adj.  $p<0.001$  in all pairwise comparisons). Thus, we tested if the recovery factor discriminates LC in both males and females by repeating our associations with LC status in the Test cohort separated by sex. Recovery factor scores were significantly associated with the binary assignment to LC vs. MIN groups in females but not in males after p-value adjustment (Figure S7B), although the trend of lower scores in LC participants persisted in males. When considering individual PRO groups, recovery factor scores discriminated between MIN vs. COG and MIN vs. MLT groups for females and MIN vs. MLT PRO groups for males (Figure S7C). Given that the LC incidence is lower in males, it is notable that recovery factor scores were generally higher in males versus females regardless of LC status. Geometric mean scores of the leading-edge analytes in the recovery factor from the heme metabolism and androgenic steroid pathways and the significant SPEAR analytes lost significance in one or both sexes when the cohort was divided into males and females (Figure S7D). Notably, though, their combined score remained significantly associated with LC in both sexes (Figure S7D).

### **Vaccination is not associated with altered recovery factor scores**

Our cohort was enrolled prior to the national SARS-CoV-2 vaccine rollout for the general population. During the longitudinal post-hospitalization follow-up period, as vaccines became broadly available, close to 75% of the participants in the IMPACC Convalescent cohort received a SARS-CoV-2 vaccination (Figure S8, A and B). To assess the potential influence of the vaccine response on the immune profiling data and thus the recovery factor, we compared recovery factor scores per visit for events occurring before and after the first vaccination dose, as well as events

occurring within a three-week period after any vaccination dose, when vaccine responses have been shown to impact immune profiles (68,69). No significant difference was found in recovery factor scores across these comparisons, indicating a negligible effect of vaccination on immune profiles related to LC in our patient cohort (Figure S8, C and D).

### **Recovery factor scores during the acute disease phase associate with LC status during convalescence**

We next investigated whether the immune elements identified in the recovery factor were predictive of LC status when measured at the acute infection phase, prior to LC development. We computed recovery factor scores using immune profiling data from all participants in the Convalescent cohort during their acute phase visits (Visits 1 to 6, spanning hospital admission through 26-35 days post-admission). Remarkably, recovery factor scores were significantly higher in MIN versus LC participants as early as hospital admission (Visit 1) and consistently during the acute period (Figure 5A, Figure S9A). Recovery factor scores were also significantly higher in MIN versus COG groups and MIN versus PHY groups in the acute phase when assessed across the 28-day time course (Figure 5B, Figure S9B). Geometric means of heme metabolism and androgenic steroid pathway analytes from the recovery factor, as well as the 26 significant SPEAR recovery factor analytes were also significantly associated with LC status during the acute phase. The combined geometric mean score of analytes from these three feature groups in acute phase data associated most significantly with MIN versus LC status (Figure 5C), as it did previously in the convalescent phase (Figure 3C).

We further assessed whether altered circulating immune cell composition in the acute phase could contribute to acute-phase recovery factor scores. Association testing of recovery factor scores with whole blood CyTOF measurements during the acute phase showed that CD4+ and CD8+ T cells, conventional and plasmacytoid dendritic cells, eosinophils, basophils, and CD56hi

CD16low natural killer (NK) cells were significantly positively associated with the recovery factor scores. Within the CD4+ T cell compartment, naïve, central memory (TCM), and effector memory (TEM) subsets, as well as non-naïve regulatory T cells (Treg) were significantly associated with recovery factor scores, while activated CD4+ T cells were inversely correlated. Within the CD8+ T cell compartment, naïve, TCM, and TEM subsets were positively associated with recovery factor scores, as were NKT cells and CD161+ MAIT cells. In contrast, monocytes, neutrophils, B cells, and plasmablasts in the acute phase were negatively associated with recovery factor scores (Figure 5D). These findings are consistent with a previous study that found higher plasmablast counts and lower total CD4+ T, total CD8+ T, CD4+ TEM, CD8+ TEM, Treg, NK, and dendritic cell counts in immune-cell populations sampled at days 0-14 after infection in COVID-19 patients who experienced persisting symptoms at days 91-180 after infection (29). The similarities across both studies indicate an acute blood immune cell type signature of LC that is robust to variance in patient cohorts and LC definition.

In summary, our findings indicate that the major biologic signatures of the recovery factor that stratify LC from recovered participants in the convalescent phase – elevated heme metabolism gene signatures, reduced androgenic steroids, increased circulating inflammatory mediators, and increased monocytes and neutrophils – are evident early in the acute phase.

### **Acute-phase recovery factor scores distinguish acute disease severities and predict LC risk irrespective of acute severity**

We investigated the full IMPACC study cohort (n = 1,148 participants with at least one sample measurement at Visit 1 for the omics modalities included in our model) to assess whether recovery factor scores determined from acute phase data would associate with patient severity trajectory group assignments. For this analysis we included participants who did not survive beyond 28 days post-hospital admission and participants without biospecimens and/or surveys during the

convalescent phase. Recovery factor scores were significantly associated longitudinally with acute disease trajectory groups and were highest in participants with milder disease courses (TG1-TG3) and lowest in participants with the most severe acute disease trajectories (TG4 and TG5) (Figure 6A). Acute-phase recovery factor scores increased over time for participants in all trajectory groups except TG5, the most severe group in which participants died by day 28 after hospital admission (Figure 6A). To assess whether the association between acute recovery factor scores and convalescent LC status was simply due to acute recovery factor scores being an indicator of acute disease severity, we repeated the association test including trajectory group as a covariate at each visit (Figure 6B) and longitudinally (Figure S9C). LC status was still significantly associated with acute recovery factor scores even after taking trajectory group into account. These findings suggest that recovery factor scores in the acute phase contain valuable information for predicting convalescent LC status beyond its correlation with acute disease severity.

### **Machine learning models based on the recovery factor scores predict LC status during the convalescent phase**

We next assessed whether a combination of recovery factor scores and clinical characteristics (Figure S10) could improve predictive performance. Machine learning models trained on acute phase recovery factor scores performed better than those trained on clinical features, and a model trained on the combination of both performed best (Figure S11A). Similarly, Machine learning models trained on both convalescent phase recovery factor scores and clinical features performed better than models trained on either alone (Figure S11B). A model trained on the 26 SPEAR significant analytes was also predictive for LC, although at lower performance than models trained on the full recovery factor (Figure S11B). This sparse model could be advantageous in a clinical setting as a diagnostic to identify individuals with LC. Notably, recovery factor scores as early as Visit 1 provided predictive performance (Figure S11C), indicating that the recovery factor captures

early predictive features of LC during the acute phase, albeit the signal at this early time point is not as strongly predictive as later in the convalescent phase.

## DISCUSSION

In this study, we applied supervised multi-omics integration methods to identify biologic features associated with LC in 513 participants from the IMPACC cohort. We took advantage of data availability from this cohort that was followed longitudinally after hospitalization with COVID-19 through 28 days of the acute disease phase and up to one year post-discharge (18,66). The IMPACC cohort is unique in its comprehensive inclusion of clinical data, biospecimens, and quarterly patient-reported outcome surveys, combined with multi-omics immunophenotyping at multiple time points throughout the acute and convalescent disease phases. A previous study of this cohort identified demographic and clinical risk factors associated with LC development, including female sex, comorbidities such as chronic heart, lung, and neurologic diseases, and a longer hospital stay (18). Here, we analyze biologic data collected during the convalescent phase, allowing us to identify a multi-omics “recovery factor” capable of discriminating participants who recovered with minimal deficits from those who experienced clinical LC symptoms. Notably, we find that as early as 72h after hospital admission for COVID-19, recovery factor scores predict which patients, irrespective of their acute disease severity, will go on to experience LC. Biologic features associated with the recovery factor score indicate that reduced androgenic steroid levels, increased heme metabolism signatures, and persistent elevation of inflammation-associated serum proteins are hallmarks of LC that both identify individuals with LC during convalescence and predict which acute COVID-19 patients will experience LC.

Increased levels of androgenic steroids in serum were positively associated with recovery factor scores and with participants who did not experience LC. Seven of the twelve leading-edge androgenic steroid metabolites, were also included within the list of 26 analytes that were statistically significant within the recovery factor. Limited studies have elucidated the role of reduced androgenic steroids in LC, but in agreement with our findings, lower testosterone levels



have been associated with increased LC symptomatology in both males and females (31). Several intermediate metabolites in the canonical steroid hormone biosynthesis pathway were associated with the recovery factor and were decreased in LC participants, including sulfated forms of testosterone precursors (pregnenolone and DHEA) and downstream metabolites (androsterone, epiandrosterone, and 5alpha-androstan-3beta,17beta-diol). DHEA-S, a leading-edge gene in the androgenic steroid pathway and a significant analyte in the recovery factor, exhibits immunosuppressive and anti-inflammatory effects, particularly in neuroinflammation (72). In addition, testosterone can play an immunomodulatory role and is often reduced in patients with other critical illnesses (73). Thus, lower androgenic steroid levels may contribute to persistent inflammation in LC.

The list of top androgenic steroid metabolites in the recovery factor overlaps with the androgenic steroid signature from an all-female cohort of healthy controls compared to myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) patients (70). All six androgenic steroid metabolites that were significantly elevated in healthy controls in the ME/CFS study (DHEA-S, androstenediol (3alpha, 17alpha) monosulfate (2), androstenediol (3beta,17beta) disulfate (2), 5alpha-androstan-3beta,17alpha-diol disulfate, androsterone sulfate, epiandrosterone sulfate) (70) were also part of the 26 significant SPEAR analytes, strongly implicating this signature in the shared symptomology, such as fatigue, post exertional malaise, and sleep disturbances, between ME/CFS and LC (71). In our study, a geometric mean score consisting of leading-edge metabolites from the androgenic steroids pathway significantly differentiated MIN vs. LC in the entire cohort. Although ME/CFS criteria were not evaluated in our cohort, in part because similarities between LC and ME/CFS were not evident in 2020 when the cohort was enrolled, future studies could investigate whether features of the recovery factor are predictive for ME/CFS.

The heme metabolism transcriptional signature in PBMCs was inversely associated with the recovery factor, such that it was elevated in LC participants during the convalescent period. As with androgenic steroids, the leading-edge genes of the heme metabolism pathway were also higher during acute COVID-19 in participants who would later experience LC. Notably, overexpression of a heme metabolism signature in blood was recently reported in a separate cohort of 102 participants, including non-hospitalized and hospitalized COVID-19 patients evaluated 1-3 months after infection (29) as well as in a cohort of non-hospitalized COVID-19 patients (63), demonstrating that elevated heme metabolism is a common signature of LC across patients with diverse acute COVID-19 disease severities. In Hanson et al. (29), elevated heme metabolism was related to stress erythropoiesis induced by inflammation-associated anemia driven by IL-6-mediated hepcidin upregulation (29,74). Participants who experienced LC had reduced iron and HGB 2-weeks to 1-month after COVID-19 infection. This iron restriction was proposed not only to induce anemia, but also to impair lymphocyte function, which could delay resolution of acute infection, resulting in sustained inflammation and thus persistent anemia that may partially explain the systemic symptomatology of acute COVID-19 and LC. Anemia of inflammation, also known as anemia of chronic disease, is a common complication associated with chronic inflammatory illnesses as well as ICU admission (75). In our study, anemia at hospital discharge was significantly negatively associated with the recovery factor, consistent with the prior finding that anemia as a complication was associated with LC (18). Moreover, HGB and hematocrit measurements at hospitalization showed a significant positive association with the recovery factor. Notably, expression levels of the leading-edge heme metabolism genes we identified in our study could also predict LC participants in the two independent cohorts discussed above (29,63), validating the relevance of the heme metabolism signature identified in the recovery factor, and demonstrating its ability to predict which patients will experience LC as early as the acute phase of COVID-19. Anemia status prior to infection was not available for the IMPACC cohort, nor was it assessed in the two cohorts in which we validated our heme

metabolism signature (29, 63). Interestingly, an assessment of LC and pre-existing comorbidities, identified by self-reported questionnaires, found that pre-existing anemia was associated with decreased risk of developing LC (76). This finding is consistent with a model in which SARS-CoV-2 infection drives the inflammation that induces anemia associated with LC. However, future studies are needed to evaluate whether pre-existing anemia is a risk or protective factor for LC.

We also find evidence of persistent inflammation in LC participants. Inflammation-associated serum factors, such as CXCL9, CSF1, and FGF21, were identified by SPEAR as significant analytes in the recovery factor, albeit they did not reach significance when associated individually with LC status. Of note, FGF21 measured in the acute phase was previously associated with cognitive and multidomain deficit PRO clusters relative to MIN in this cohort (18) and has been proposed as a biomarker for chronic inflammation in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)(77), a complex chronic disease that overlaps clinically with LC. Furthermore, LRG1, which is activated by the inflammatory IL-6/STAT3 pathway is significantly elevated in LC participants, perhaps contributing to vascular pathology in LC (55–57). Although IL-6 did not reach significance in the SPEAR factor (SPEAR Bayesian posterior selection probability = 0.86), it was within the top 50 analytes, perhaps promoting anemia and increased heme metabolism signatures, as discussed above. Also, recovered participants demonstrated elevated levels of the tissue repair protein DNER, consistent with other cohorts (28). Together, these findings point to inefficient tissue repair, as well as persistent inflammation, driving anemia and stress erythropoiesis as key drivers of LC.

Across all time points, consistent immune cell subsets were associated with the recovery factor. In both the acute and convalescent phases, CD161+ MAIT cell frequencies were positively associated with the recovery factor, while monocyte, neutrophil, and CD14+ CD16- classical monocyte frequencies were negatively associated with the recovery factor. CD14+ monocytes

can induce CD8<sup>+</sup> T cells to produce high levels of IFN- $\gamma$  in LC patients (78). The consistent association of classical monocytes with low recovery factor scores suggests that they contribute to inflammation starting in acute disease and continuing into convalescence in participants who experience LC. In contrast, the B cell population was negatively associated with the recovery factor during acute disease, but positively associated during convalescence. A prior study with limited participants reported successful treatment of LC with intravenous IgG (IVIG) administration (79), suggesting that restoring homeostasis of B cells and subsequent IgG production should be evaluated for treating LC. An ongoing clinical trial evaluating IVIG for treatment of neurological LC ([NCT05350774](#)) will provide further insights. Our data on immune cell frequencies in the recovery factor agree with several previous reports of LC subjects. The positive association of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, plasmacytoid DCs, and conventional DCs with the recovery factor during acute disease, with a negative association of monocytes, neutrophils, and B cells indicates reduced T cell immunity relative to inflammatory innate immunity in individuals susceptible to LC. This finding is in keeping with cellular trends observed in Hanson et al. in the acute disease (29) and in males during convalescence by Silva and colleagues (31). Consistent with Klein et al. (26), we do not find significant associations of the recovery score with naive CD4<sup>+</sup> or naive CD8<sup>+</sup> T cells in the convalescent phase of COVID-19. Though whole blood single-cell RNAseq data was not available for this cohort, transcriptomics profiles of individual cell subsets, such as monocytes and neutrophils, could provide further insight into the pathways by which they contribute to LC.

A previous study from IMPACC identified a ‘severity factor’ that significantly associated with clinical outcomes during acute COVID-19 (39). Given that both the severity factor and the recovery factor are associated with inflammatory signatures during acute COVID-19, we compared immune cell types associated with the recovery factor to those associated with the severity factor at this stage of disease. There were notable similarities: for example, monocytes, B cells, and neutrophils are negatively associated with the recovery factor and positively

associated with the severity factor. Likewise, CD4+ and CD8+ T cells are positively associated with the recovery factor and negatively associated with the severity factor. These cellular associations suggest that inefficient adaptive immunity during acute disease, with elevated frequencies of inflammatory innate cells contribute to LC susceptibility. This model is consistent with our past (18) and current findings in the IMPACC cohort that reduced anti-SARS-CoV-2 antibody levels and increased viral titers in patients within the first 72 hours of hospital admission are associated with participants who will experience LC (Figure S10, B and C). These findings are also consistent with other reports that hospitalized individuals are more susceptible to LC than non-hospitalized individuals (14,80). Nonetheless, our recovery factor predicts which patients will experience LC irrespective of acute disease severity, indicating that the model has learned features of COVID-19 beyond inflammation that are associated with COVID-19 severity.

Although symptom groups, such as respiratory symptoms, were found to be significantly associated with LC in this cohort (18), the multi-omics recovery factor does not associate with a particular clinical symptom group. Instead, it captures biomarkers predictive of global physical deficits, as reported by patients after acute COVID-19 disease. While assessing the entire multi-omics SPEAR factor in convalescent patients is impractical, our findings indicate that assessing the 26 significant SPEAR analytes would aid in LC diagnoses (Figure S11B). Future studies with even larger datasets would be needed to identify LC mechanisms specific for distinct endotypes of disease.

This study has several limitations. The reliance on self-reported survey data to identify symptoms and classify participants into MIN/LC and individual PRO clusters may introduce potential biases. To address this, population-normalized PRO survey scores were utilized (81–83), and comparisons were made to pre-illness health status when possible. Additionally, our study was designed early in the pandemic (March 2020), before the full spectrum of LC symptoms was

characterized, and thus the surveys did not capture current commonly recognized manifestations such as brain fog, fatigue, sleep disturbances, neuropathy, and dysautonomia. Patients were also not evaluated clinically for measures of pain, sensory sensitivity, or other clinical features of chronic disease during convalescence, when surveys were instead administered, so we cannot determine if the recovery factor correlates with clinical features of chronic disease. Self-selection bias may also be present, as patients with severe LC symptoms might have been less likely to respond to the surveys. As the study cohort was recruited during early phases of the pandemic (May 2020 through March 2021), it consists of individuals infected only with the original SARS-CoV-2 strain and does not include data on subsequent variants of concern; however, we note that the recovery factor Heme metabolism signature identified LC patients in a cohort infected with the Omicron variant (63)(Figure S6D). Vaccination data were self-reported and limited to the post-acute phase since enrollment was largely completed prior to vaccine rollout, and exact vaccination dates were unavailable for some participants. Furthermore, as part of the study design, all participants in the IMPACC cohort were hospitalized for COVID-19. Consequently, the multi-omics factors were constructed without incorporating profiles from COVID-19 patients with mild disease, potentially introducing a bias towards those with severe disease. Nonetheless, we note that participants who recovered after hospitalization without experiencing LC (MIN group) have a median Physical PRO score that is slightly better (>50) than the overall population norm (Figure S3A), indicating that the severe nature of disease experienced by this hospitalized cohort did not bias health in the convalescent period to worse than population norms. We are not aware of a dataset from a non-hospitalized or asymptomatic cohort with the same depth of OMICs measurements needed to reconstruct the entire recovery factor to test if predictive power is maintained in non-hospitalized cohorts. However, we validated that elevated heme metabolism scores, a key biologic pathway in the recovery factor, were associated with LC in a cohort that included hospitalized and non-hospitalized COVID-19 patients (29), as well as in a cohort consisting of non-hospitalized COVID-19 patients (63). While it remains possible that biologic

signatures of LC exclusive to COVID-19 patients with mild acute disease may not be included in the recovery factor, validation of the heme metabolism signature in these independent cohorts suggests that the molecular signatures of LC identified in our hospitalized cohort likely extend to non-hospitalized and asymptomatic SARS-CoV-2 infected individuals.

Despite these limitations, the study possesses multiple strengths. In addition to the prospective design, with acute and convalescent longitudinal multi-omics profiling, enrollment through multiple sites across the US enhances the broad representation of the cohort and mitigates potential participant recruitment biases, contributing to the robustness of the findings.

In conclusion, supervised multi-omics factor construction of immune profiling data from SARS-CoV-2 infected participants who recovered with minimal deficits or experienced LC indicates that peripheral blood leukocytes and serum factors associated with inflammation, reduced androgenic steroids, and elevated heme metabolism signatures predict which participants will experience LC, irrespective of acute disease severity. Moreover, these signatures are maintained into convalescence, indicating that persistent inflammation driving anemia is likely a key contributor to LC. Further studies will be needed to determine why inflammation persists in some COVID-19 patients. We did not assess persistent SARS-CoV-2 viral loads or viral reactivation; however, a recent IMPACC study (84) suggests latent virus reactivation may contribute to LC, consistent with other studies (26,30,31). Altogether, our data, paired with prior congruent reports, suggest that impaired lymphocyte function early in COVID-19 reduces cellular and humoral adaptive immunity and contributes to high SARS-CoV-2 viral loads. Elevated viral loads can trigger innate immune cell responses that increase inflammatory cytokines, driving inflammation-associated anemia that further reduces lymphocyte function, which could enable reactivation of latent viruses. Such unresolved persistent inflammation likely leads to LC pathology. Strategies to break the cycle of

inflammation and correct the inflammation-associated anemia may promote recovery from LC and merit further investigation.

## **METHODS**

### **Sex as a biological variable**

Our study examined male and female participants. The cohort included 310 (60%) men and 213 (40%) women. Female sex was negatively associated with the recovery factor, in line with a higher proportion of women experiencing LC. To control for this imbalance, sex and age were used as covariates in statistical testing to identify robust trends for both sexes unless otherwise stated.

### **Study design**

The IMPACC Cohort consists of 1,164 hospitalized COVID-19 patients from 20 U.S. hospitals (15 academic institutions), enrolled within 72 hours of admission between May 2020 and March 2021. Participants with confirmed positive SARS-CoV-2 PCRs were followed during the acute infection phase (1–28 days post-admission) and convalescent phase (3–12 months post-discharge). Clinical data (e.g. hospital stay, comorbidities, complications, mortality) and biological samples were collected during the acute phase, while standardized patient-reported outcome surveys were assessed quarterly over the convalescent phase (18,37,38). Six validated surveys, including the Patient-Reported Outcomes Measurement Information System (PROMIS) Physical function, Cognitive function, Global Health Mental, Psychosocial Illness Impact and Dyspnea Time extension (80,81), and the EQ-5D-5L (82) were used to evaluate general health and deficits in specific health domains. Also, overall health recovery scores compared post-discharge function to pre-infection status (18). Full study design details are available in the Supplemental Methods.

### **Statistics**

Unless otherwise stated, multiple comparisons were accounted for via Benjamini-Hochberg correction, with adj. p-values of less than 0.05 considered significant. Linear mixed effects



modeling was used for differential analysis of multi-omics factors, analyte geometric means, and individual analytes across LC groups, after adjusting for sex, age and visit number as fixed effects and enrollment site as a random effect. Generalized mixed-effects modeling was utilized to investigate longitudinal variations across conditions using samples from all visits after further adjusting for participant ID as a random effect. These sets of fixed and random effects for baseline and longitudinal samples were the default for all mixed-effects modeling, unless otherwise stated. See Supplemental Methods for detailed descriptions of all statistical analyses and models.

### **Study approval**

NIAID staff conferred with the Department of Health and Human Services Office for Human Research Protections (OHRP) regarding the potential applicability of the public health surveillance exception [45CFR46.102 (l) (2)] to the IMPACC study protocol. OHRP concurred that the study satisfied criteria for the public health surveillance exception, and the IMPACC study team sent the study protocol, and participant information sheet for review and assessment to institutional review boards (IRBs) at participating institutions. Twelve institutions elected to conduct the study as public health surveillance, while 3 sites with prior IRB-approved biobanking protocols elected to integrate and conduct IMPACC under their institutional protocols (The University of Texas at Austin, IRB 2020-04-0117; University of California San Francisco, IRB 20-30497; Case Western Reserve University, IRB STUDY20200573) with informed consent requirements. Participants enrolled under the public health surveillance exclusion were provided information sheets describing the study, samples to be collected, and plans for data de-identification and use. Those who requested not to participate after reviewing the information sheet were not enrolled. In addition, participants did not receive compensation for study participation while inpatient and subsequently were offered compensation during outpatient follow-ups.

## **Data availability**

Data used in this study is available on the ImmPort repository under accession number SDY1760 and in the Database of Genotypes and Phenotypes (dbGaP) under the accession number phs002686.v2.p2. All code used in this study is deposited on Bitbucket (<https://bitbucket.org/kleinstein/impacc-public-code/src/master/multiomics-longcovid/>). The raw data for all figures are available in the Supporting Data Values file.

## **AUTHOR CONTRIBUTIONS**

PMB, SHK, LG, LIRE conceived the study. AH, CS, BP, JD-A curated the data. GG, JM, JPG, JFM, AH, CS, TC, NDJ conducted formal analysis. The IMPACC Network acquired funding and provided resources. GG, JM, JPG, JFM, SHK, LG designed the study methodology and code. SF, MCA, OL, KKS, THH, RRM, JD-A, SHK, LG, LIRE supervised the study. All authors wrote, edited, and reviewed the manuscript. Co-first authorship was assigned based on contributions.

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## **SUPPLEMENTAL MATERIAL**

Supplemental material includes experimental model and study participant details, method details, supplemental figures S1-10, Supplemental Acknowledgements and conflicts of interest.

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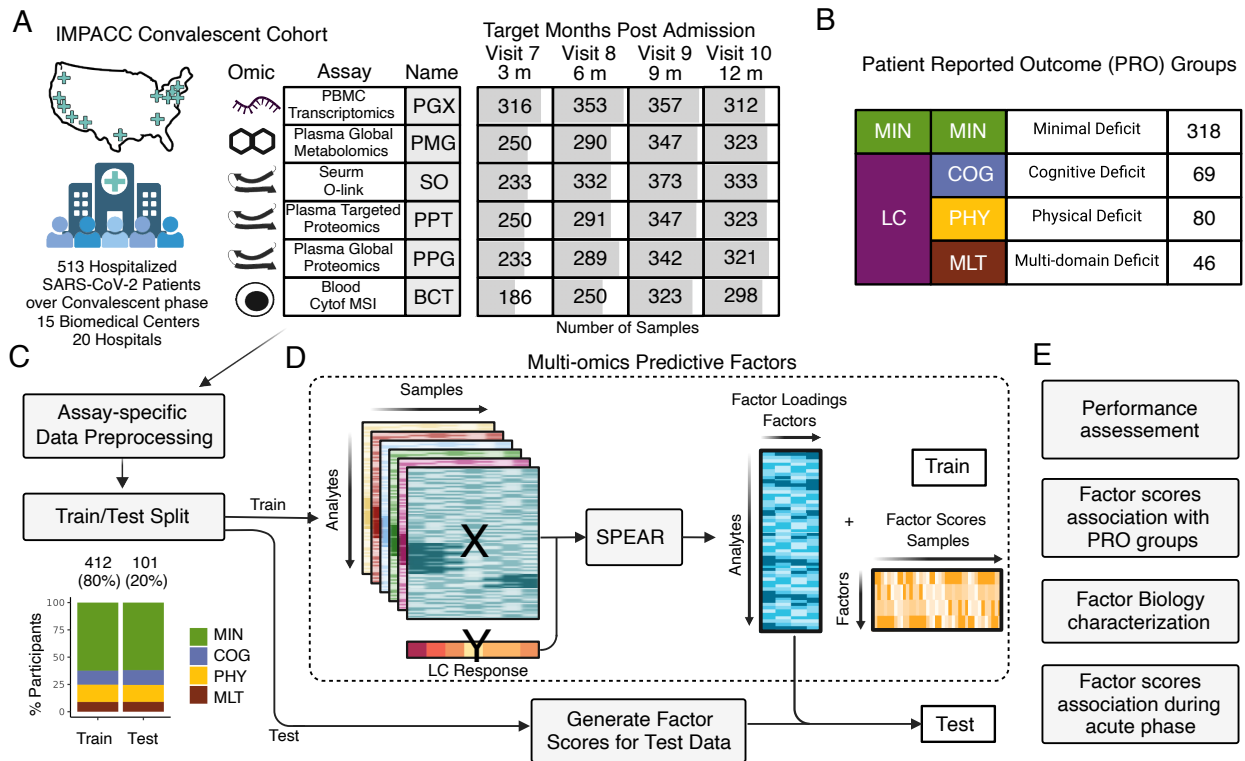
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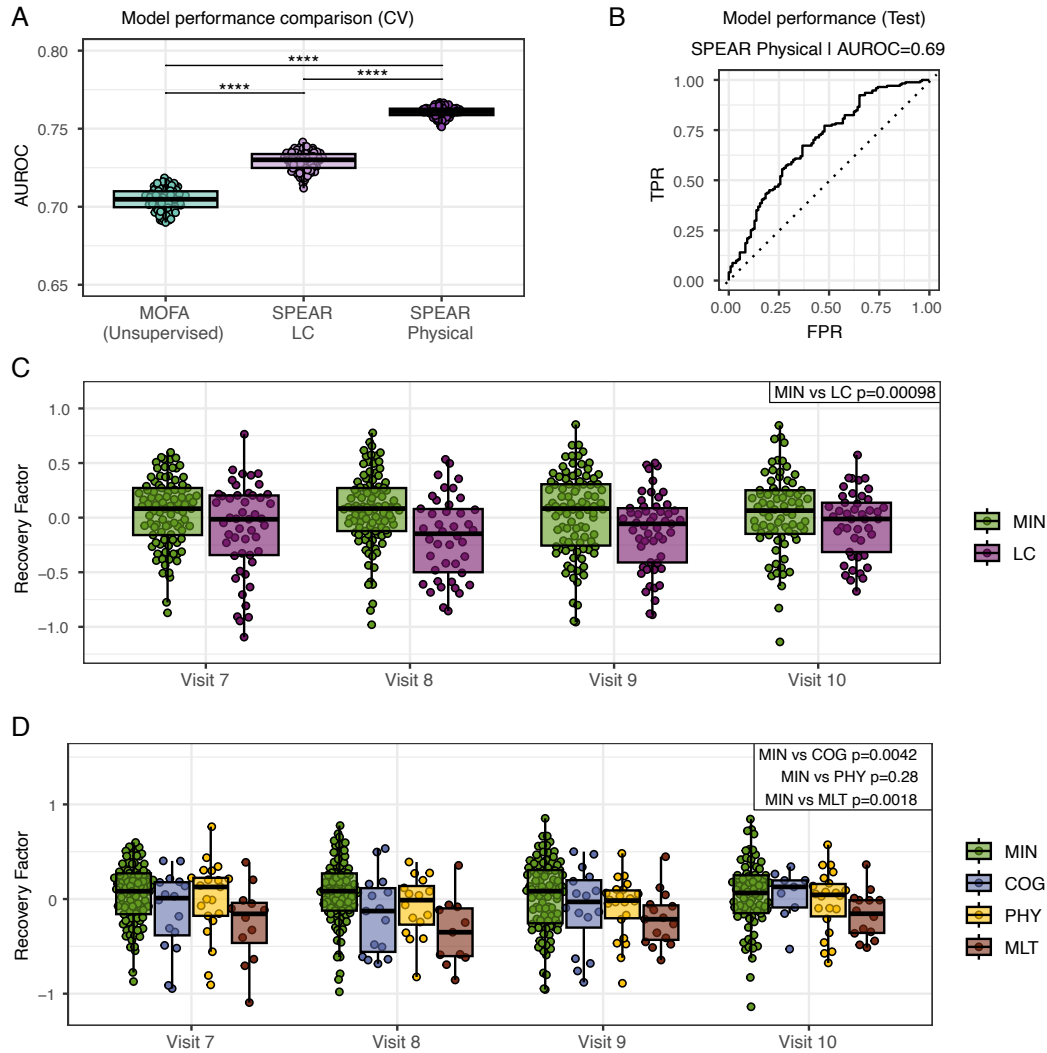
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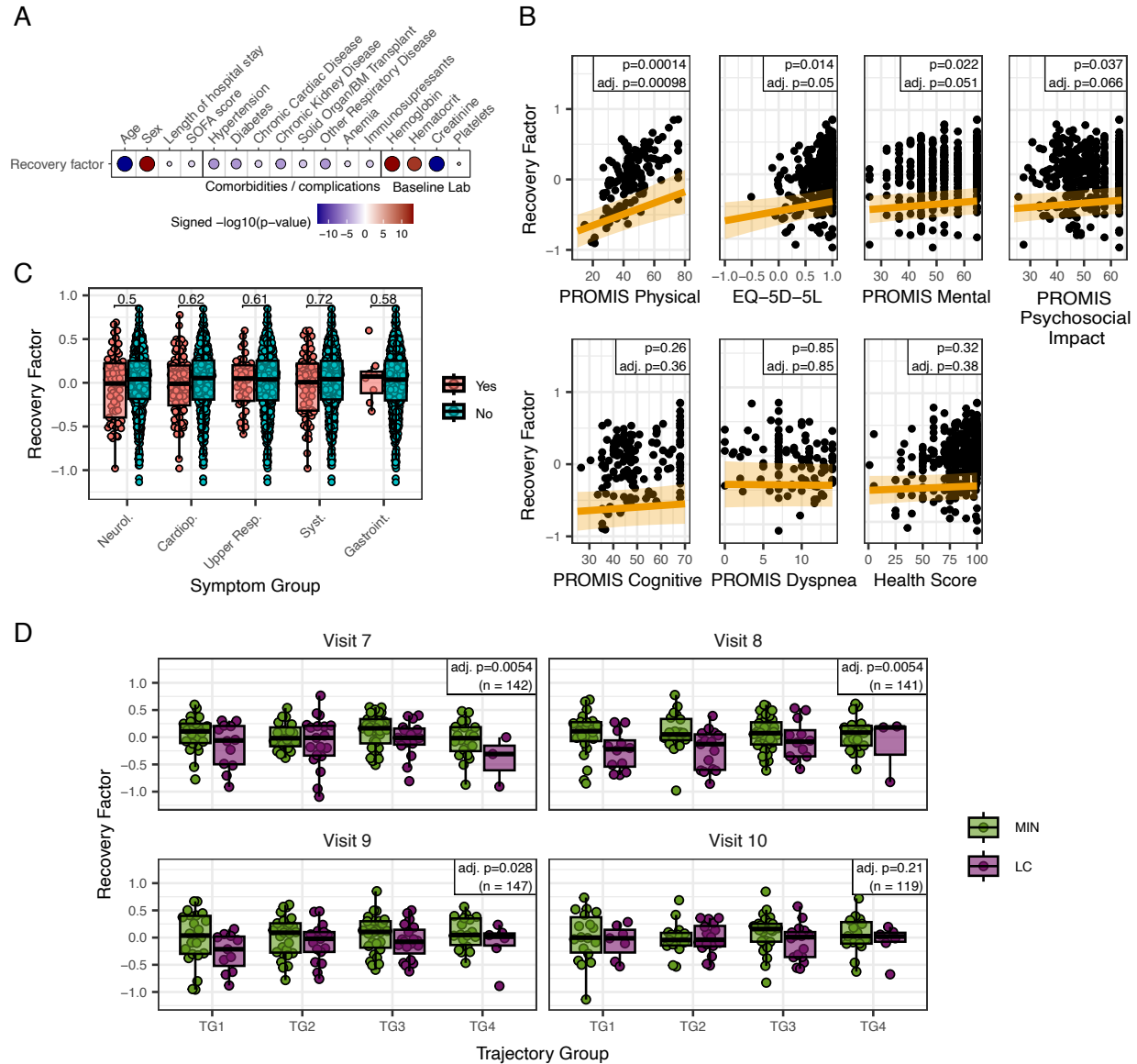


**Figure 1. Multi-omics data overview and generation of a predictive LC factor. (A)** Number of samples used in the multi-omics data integration strategy by assay (rows) and scheduled time of collection (columns). Shading indicates the frequency of samples with data availability at the indicated visit. **(B)** Patient classification in Patient Reported Outcome (PRO) clusters according to the PRO survey scores (18). **(C)** Individual assay data were preprocessed and split into Train and Test cohorts by participant in an 80/20 split, maintaining the proportion of PRO cluster participants in each partition. **(D)** Preprocessed assay data and LC response outcomes for the Train cohort were used to identify multi-omics predictive factors with SPEAR. Factor scores were then calculated for the Test cohort. **(E)** The performance of the multi-omics predictive factors to classify patients into presence and absence of LC was assessed on the Train cohort via cross-validation and then validated on the Test cohort. The predictive factor scores were confirmed to be associated with LC after correcting for possible confounding variables. In depth analysis of enriched biological pathways and significant analytes relevant for the prediction was performed. Factor scores were computed for the acute infection immune profiles, and association analysis with LC at these early time points was performed. See also Figures S1 and S2.



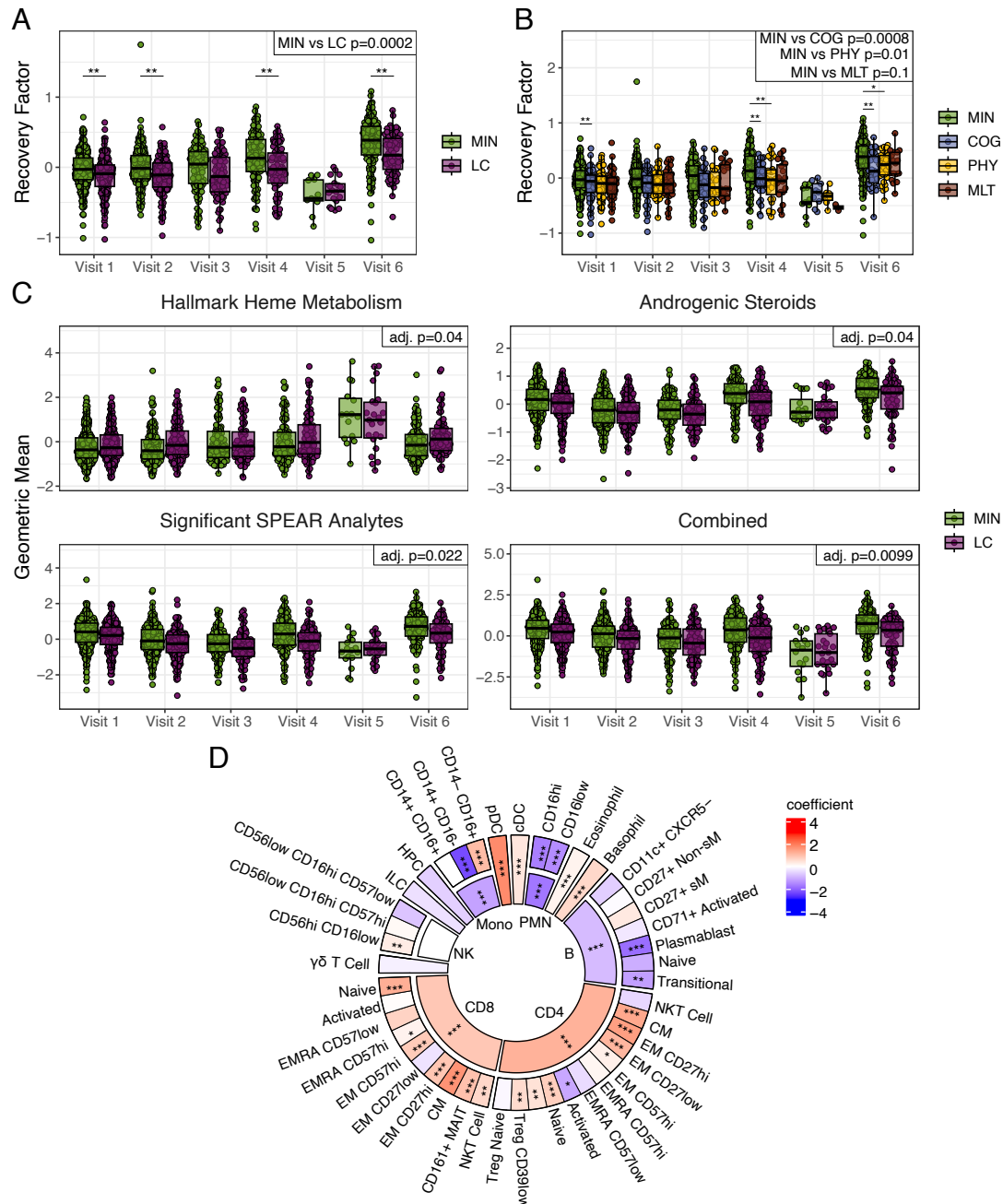
**Figure 2. Identification of a convalescent multi-omics recovery factor that discriminates long COVID.** (A) Predictive performance of a lasso model trained on the MOFA and SPEAR factors to discriminate LC vs MIN at the event level. The mean AUROC of a 10-fold cross-validation on the Train Cohort, for 100 bootstrapped model training repetitions are shown. Significance was calculated by standard normal approximation of bootstrapped differences between models (t-test, \*\*\*\*adj. p-value  $\leq 0.0001$ ) (B) Predictive performance of the SPEAR Physical model to discriminate LC vs MIN on the Test cohort. ROC curve of model (solid line), random classifier (dashed line), and AUROC value are shown. TPR: true positive rate, FPR: false positive rate. (C) Recovery factor scores for the Test cohort of the MIN and LC groups at 3 months (Visit 7), 6 months (Visit 8), 9 months (Visit 9) and 12 months (Visit 10) after hospital discharge. (D) Recovery factor scores of the individual PRO clusters by visit for the Test cohort. P-values in C and D show the significance of the recovery factor score association with MIN vs LC and pairwise PRO cluster combinations, respectively (see methods for association details). See also Figure S3, S4, and S5.





**Figure 4. Associations of clinical measurements with recovery factor scores. (A)** Association of recovery factor scores with clinical features (demographics, comorbidities, complications and baseline lab measurements). Dot plot shows the signed adjusted p-values indicating the clinical feature term significance from a linear mixed-effect model with enrollment site and participant as random effects to explain the convalescent phase recovery factor scores. Sex and discretized age were further adjusted as fixed effects for clinical features other than sex and age. Only significant associations (adj. p-value <0.05) are shown. **(B)** Associations of recovery factor scores with individual PRO survey scores (PROMIS scale scores, EQ-5D-5L and health score) in the test cohort. Raw and adjusted p-values indicated the PRO score term significance in linear mixed effect models. **(C)** Associations of recovery factor scores with each indicated symptom group in the test cohort: Neurological, Cardiopulmonar, Upper respiratory, Systemic, Gastrointestinal. Numbers are the uncorrected significance (p-values) of the symptom group term in linear mixed effect models. **(D)** Recovery factor scores per participant in the test cohort, separated into MIN and LC groups by acute phase trajectory groups, stratified by visit. P-

values for panels B-D show the endpoint term of a linear mixed effect model with sex, discretized admit age, and trajectory group as fixed effects and enrollment site as random effect. No individual MIN vs. LC comparisons were significant after p-value correction (\* adj. p-value < 0.05, \*\* adj. p-value < 0.01, \*\*\* adj. p-value < 0.001).

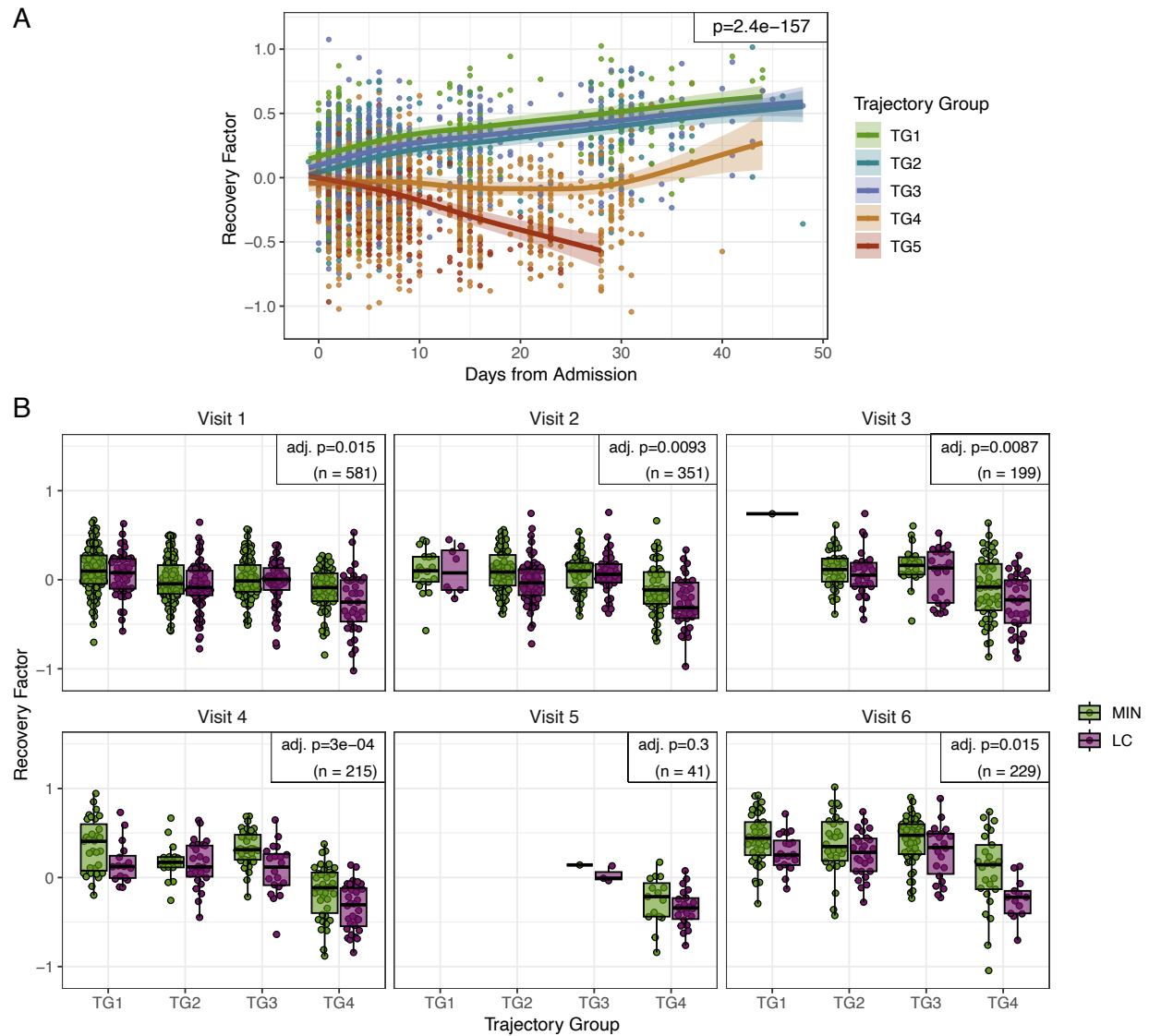


**Figure 5. Recovery factor scores in acute phase data associate with eventual LC status.**

**(A)** Recovery factor scores during the acute disease phase for participants in the LC and MIN groups within 72h of hospital admission (Visit 1) and at day 4 (Visit 2), day 7 (Visit 3), day 14 (Visit 4), day 21 (Visit 5), and day 28 (Visit 6) after admission. **(B)** Recovery factor scores during the acute disease phase for participants in individual PRO clusters. **(C)** Geometric mean of analytes in enriched gene and metabolic sets and/or significant SPEAR analytes during the acute phase. No individual per-visit comparisons were significant after p-value correction. P-values in top-right box in A-C show the significance of the recovery factor score or geometric mean signature association with MIN vs LC or pairwise PRO cluster combinations. Bars above the boxplots show the pairwise significance across groups in a per-visit comparison (\*\*  $p<0.01$ , \* $p<0.05$ ). **(D)** Recovery factor scores association with whole blood CyTOF immune cell populations during the

acute phase (\* adj. p-value < 0.05, \*\* adj. p-value < 0.01, \*\*\* adj. p-value < 0.001). See also Figure S9.





**Figure 6. Recovery factor scores associate with acute disease phase trajectory groups, but identify LC irrespective of acute severity. (A)** Longitudinal analysis of acute recovery factor scores for the full IMPACC cohort stratified by trajectory group (N=1,148 participants). P-value shows the significance of the trajectory group term in a longitudinal model correcting for age and sex as fixed effects and enrollment site and participant ID as random effects. **(B)** Recovery factor scores in the acute phase by convalescent MIN/LC label, stratified by acute trajectory group and visit number. P-values show significance in distinguishing MIN vs. LC labels in linear mixed models with sex, discretized admit age, and trajectory group as fixed effects and enrollment site and participant ID as random effects, performed separately for each acute visit and corrected across all visits.