



UNIVERSITY OF
LATVIA

Summary of
Doctoral Thesis

Laura Ansone

**LONGITUDINAL
MULTI-OMICS PROFILING
OF SEVERE COVID-19
AND POST-ACUTE RECOVERY**

Riga 2026



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FACULTY OF MEDICINE AND LIFE SCIENCES

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AND POST-ACUTE RECOVERY**

SUMMARY OF DOCTORAL THESIS

Submitted for the degree of Doctor of Biology
Subfield of Molecular Biology

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Form of the thesis: a collection of research papers in the field of Biology, subfield of Molecular Biology.

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ABSTRACT

Coronavirus disease 2019 (COVID-19) presents with diverse acute manifestations and may lead to long-term complications, known as Long COVID or post-acute sequelae of SARS-CoV-2 infection. Although acute COVID-19 pathogenesis has been extensively studied, the mechanisms linking severe infection with incomplete recovery remain unclear. This doctoral thesis aimed to characterize longitudinal molecular alterations in hospitalized patients with severe COVID-19 and identify metabolic, immune, vascular, and clinically measurable markers associated with recovery and Long COVID risk.

A longitudinal omics design was used to follow hospitalized patients from acute disease through early and later recovery. Targeted serum LC-MS metabolomics revealed pronounced amino acid metabolism dysregulation at hospital admission, involving the tryptophan-kynurenine pathway, arginine-related metabolism, and glutamine depletion, indicating systemic immunometabolic activation. NMR-based plasma metabolomics identified persistent disturbances in lipid, lipoprotein, glycoprotein-associated inflammatory, and energy metabolism markers during recovery, suggesting incomplete metabolic normalization in some patients.

The central multi-omics analysis integrated whole-blood transcriptomics, urine metabolomics, kidney injury biomarkers, and clinical data. Immunothrombosis emerged as a dominant biological axis in severe COVID-19, linking complement activation, neutrophil extracellular trap formation, platelet activation, coagulation signaling, mitochondrial stress, and kidney tubular injury. Although many acute-phase processes partially normalized, patients who later developed Long COVID retained a distinct three-month blood transcriptional signature marked by persistent endothelial-associated activation, platelet reactivity, complement regulation, low-grade inflammation, and tissue remodeling.

Overall, severe COVID-19 appears to be a systemic immunometabolic and vascular disease with persistent molecular consequences in a subset of hospitalized patients. Longitudinal multi-omics profiling distinguishes acute infection-related responses from post-acute alterations and identifies candidate markers, including VWF, PROS1, complement-related markers, inflammatory glycoproteins, lipid parameters, and kidney injury biomarkers, for future risk stratification and monitoring after severe COVID-19.

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ABBREVIATIONS

<i>AKI</i>	acute kidney injury
<i>ALT</i>	alanine aminotransferase
<i>Apo-A1</i>	apolipoprotein A1
<i>ARDS</i>	acute respiratory distress syndrome
<i>AST</i>	aspartate aminotransferase
<i>BMI</i>	body mass index
<i>CFH</i>	complement factor H
<i>COVID-19</i>	coronavirus disease 2019
<i>CRP</i>	C-reactive protein
<i>CXCL5</i>	C-X-C motif chemokine ligand 5
<i>EHR</i>	electronic health registry
<i>FDR</i>	false discovery rate
<i>FECH</i>	ferrochelataase
<i>FSTL1</i>	folistatin-like 1
<i>Glyc</i>	total glycoprotein acetylation signal
<i>GlycA</i>	glycoprotein acetylation signal A
<i>GlycB</i>	glycoprotein acetylation signal B
<i>GST-α</i>	glutathione S-transferase alpha
<i>HDL</i>	high-density lipoprotein
<i>HIF1A</i>	hypoxia-inducible factor 1-alpha
<i>ICD-10</i>	International Classification of Diseases, 10th Revision
<i>IDO1</i>	indoleamine 2,3-dioxygenase 1
<i>IP-10</i>	interferon gamma-induced protein 10
<i>KIM-1</i>	kidney injury molecule 1
<i>LC-MS</i>	liquid chromatography-mass spectrometry
<i>LDH</i>	lactate dehydrogenase
<i>NAD+</i>	nicotinamide adenine dinucleotide
<i>NET</i>	neutrophil extracellular trap
<i>NMR</i>	nuclear magnetic resonance
<i>OPA1</i>	optic atrophy 1
<i>OXPPOS</i>	oxidative phosphorylation
<i>PASC</i>	post-acute sequelae of SARS-CoV-2 infection
<i>PCA</i>	principal component analysis
<i>PINK1</i>	PTEN-induced kinase 1
<i>PROS1</i>	protein S
<i>SARS-CoV-2</i>	severe acute respiratory syndrome coronavirus 2
<i>TCA</i>	tricarboxylic acid
<i>TIMP-1</i>	tissue inhibitor of metalloproteinases 1
<i>VWF</i>	von Willebrand factor

INTRODUCTION

COVID-19 is a clinically heterogeneous disease that may lead not only to severe acute illness, but also to persistent post-acute complications known as Long COVID. Although substantial progress has been made in understanding acute SARS-CoV-2 infection, the molecular mechanisms linking severe disease with incomplete recovery and post-acute complications remain insufficiently defined. Therefore, longitudinal molecular profiling is needed to distinguish transient acute responses from persistent alterations during recovery. The scientific novelty of this thesis lies in the integrated characterization of metabolic, immune, vascular, and renal molecular trajectories in hospitalized patients with severe COVID-19. The study identifies immunometabolic dysregulation, incomplete metabolic recovery, and immunothrombosis as central mechanisms associated with post-acute complications.

Aim and tasks of the study

The aim of this doctoral thesis was to characterize longitudinal molecular alterations associated with severe COVID-19 and post-acute recovery, and to identify molecular pathways and biomarkers linked to Long COVID risk.

To achieve this aim, the following tasks were defined:

1. To characterize serum amino acid metabolism alterations in hospitalized COVID-19 patients during the acute phase and recovery.
2. To evaluate longitudinal plasma metabolomic changes in severe COVID-19 patients using NMR-based metabolomics, with emphasis on lipid, lipoprotein, and energy metabolism.
3. To integrate blood transcriptomics, urine metabolomics, kidney injury biomarkers, and clinical data to identify molecular trajectories associated with recovery and post-acute complications.
4. To identify biological pathways and clinically measurable markers associated with increased Long COVID risk in hospitalized COVID-19 patients.

Theses proposed for defence

1. Severe COVID-19 is a systemic immunometabolic disease characterized by acute amino acid metabolism dysregulation, including activation of tryptophan-kynurenine metabolism, altered arginine-related metabolism, and glutamine depletion.

2. Recovery from severe COVID-19 is molecularly incomplete in a subset of hospitalized patients and is characterized by persistent disturbances in lipid metabolism, glycoprotein-associated inflammation, energy metabolism, mitochondrial stress, and renal tubular injury markers.
3. Immunothrombosis is a central biological mechanism linking severe acute COVID-19 with post-acute complications. Complement activation, neutrophil extracellular trap formation, platelet activation, coagulation signaling, and endothelial-associated activation form an interconnected molecular network during severe infection.
4. Patients who develop Long COVID after hospitalization retain a distinct three-month blood transcriptional signature consistent with persistent endothelial-associated activation, platelet reactivity, complement regulation, low-grade inflammation, and tissue remodeling.
5. Longitudinal multi-omics profiling can identify clinically relevant molecular trajectories and candidate biomarkers for post-acute risk stratification in hospitalized COVID-19 patients, including VWF, PROS1, complement-related markers, inflammatory glycoproteins, lipid parameters, and kidney injury biomarkers.

Approbation of research

The research was carried out at the Latvian Biomedical Research and Study Centre from 2020 to 2026 and was supported by the State Research Programme project No. VPP-COVID-2020/1-0016, the European Regional Development Fund project No. 1.1.1.1/21/A/029 POST-COVID-TRACK, and the European Union's Recovery and Resilience Mechanism project No. 5.2.1.1.i.0/2/24/I/CFLA/001. The results of the thesis were approbated in three scientific publications, including two published articles and one manuscript accepted in principle, and were presented at five international or scientific conferences. The doctoral thesis is prepared as a collection of research papers in Biology, subfield of Molecular Biology. The thesis comprises 119 pages and consists of an introduction, four chapters with subchapters, conclusions, theses, approbation of research, acknowledgements, and references; the thesis has no appendices.

LITERATURE REVIEW

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), developed into one of the largest global public health emergencies of recent decades. The clinical course of COVID-19 ranges from mild respiratory symptoms to severe pneumonia, ARDS, sepsis, thromboembolic complications, and multi-organ failure. According to the WHO clinical framework, severe and critical COVID-19 may involve hypoxia, ARDS, septic shock, or multi-organ dysfunction (World Health Organization, 2025). Thus, severe COVID-19 is a systemic disease involving immune dysregulation, endothelial injury, coagulation abnormalities, metabolic reprogramming, and organ-specific damage.

A major challenge after acute SARS-CoV-2 infection is the persistence or development of long-term symptoms. Long COVID, also referred to as post-acute sequelae of SARS-CoV-2 infection (PASC), is generally defined as persistent or new symptoms lasting for months after acute infection without an alternative explanation. It is clinically heterogeneous and may involve fatigue, dyspnea, sleep disturbance, chest pain, cognitive symptoms, cardiovascular and musculoskeletal manifestations, metabolic disturbances, and renal complications. Because PASC affects multiple organ systems, mechanistic understanding beyond symptom-based diagnosis is needed (Davis et al., 2023; Nalbandian et al., 2021). It remains a continuing burden for patients and healthcare systems, affecting approximately 10–20% of infected individuals, with higher incidence among previously hospitalized patients (Wulf Hanson et al., 2022).

Electronic health record-based studies have been especially important for defining post-acute phenotypes at population scale. Zang et al. (2023) used large EHR cohorts to characterize Long COVID and identified broad post-acute manifestations, including pulmonary, cardiovascular, neurological, musculoskeletal, metabolic, and general systemic complications (Zang et al., 2023). This type of data-driven phenotyping is relevant for hospitalized cohorts, where post-acute diagnoses can be used to stratify patients into recovery and Long COVID groups. In the multi-omics part of this thesis, EHR-based outcome stratification was applied using a PASC diagnosis list from Zang et al., enabling the comparison of patients with documented post-acute complications and patients who recovered without new PASC diagnoses.

Several overlapping mechanisms have been proposed to contribute to Long COVID. Highly cited reviews about Long COVID pathogenesis and molecular mechanisms, describe persistent immune activation, autoimmunity, viral persistence or antigen persistence, latent virus reactivation, dysregulated

interferon responses, microbiome alterations, mitochondrial dysfunction, and tissue damage caused during acute disease as contributing processes for long-term complications (Choutka et al., 2022; Davis et al., 2023; Nalbandian et al., 2021). These mechanisms are unlikely to act independently. Instead, Long COVID may represent several partially overlapping biological phenotypes, which helps explain why symptoms and clinical trajectories differ between patients.

Among the most relevant mechanisms for severe COVID-19 and post-acute complications is vascular dysfunction. Bonaventura et al. (2021) described endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19 (Bonaventura et al., 2021). In this model, inflammation, complement activation, neutrophil extracellular trap formation, platelet activation, and coagulation form a self-amplifying network that contributes to microvascular injury and thrombotic complications. This framework is particularly important in hospitalized patients, where acute inflammatory and thrombotic processes may leave persistent vascular consequences. Recent Long COVID research has strengthened this interpretation. Cervia-Hasler et al. (2024) showed that active Long COVID is associated with persistent complement dysregulation and signs of thromboinflammation, including complement pathway activation, altered coagulation, platelet activation, and markers of red blood cell lysis (Cervia-Hasler et al., 2024). These findings are highly relevant to the present thesis, where immunothrombosis emerged as a dominant molecular axis in hospitalized patients, and where patients who later developed Long COVID retained a three-month transcriptional signature marked by endothelial-associated activation, platelet reactivity, complement regulation, and low-grade vascular inflammation.

Multi-omics approaches are well suited to study these interconnected mechanisms. Transcriptomics can identify immune, inflammatory, platelet, endothelial, complement, and mitochondrial pathway activity; blood metabolomics can reveal functional changes in amino acid, lipid, and energy metabolism; urine metabolomics and kidney injury biomarkers can indicate systemic and renal stress. Longitudinal study designs are especially important, because they allow acute infection-related responses to be separated from persistent post-acute alterations. Accordingly, this thesis applies longitudinal blood metabolomics and transcriptomics, together with urine metabolomics, kidney injury biomarker profiling, and clinical data, to characterize severe COVID-19 as a systemic immunometabolic and vascular disease and to identify molecular trajectories associated with Long COVID risk.

MATERIALS AND METHODS

This doctoral thesis was based on a longitudinal study design aimed at characterizing molecular changes in hospitalized patients with severe COVID-19 from the acute phase through post-hospitalization recovery. Patients with confirmed SARS-CoV-2 infection were recruited in Latvia between 2020 and 2021 at Riga East University Hospital, Vidzeme Hospital, and Liepāja Regional Hospital. The study was conducted in accordance with the Declaration of Helsinki and approved by the Central Medical Ethics Committee of Latvia. Written informed consent was obtained from all participants before enrolment. Population control samples were selected from the Latvian National Biobank and used in the metabolomics studies as a pragmatic non-COVID reference group.

Biological sampling was centered on three disease stages: the acute phase at hospital admission, early recovery approximately one month after hospitalization, and later recovery three to four months after hospitalization. The first targeted serum LC-MS metabolomics study included acute and one-month recovery samples, whereas the NMR metabolomics and multi-omics studies extended the design to the three- to four-month recovery timepoint. This longitudinal structure enabled within-patient comparisons and helped reduce the influence of high inter-individual variability, which is characteristic of severe COVID-19 cohorts.

The thesis was organized as a stepwise progression of omics-based studies. First, targeted liquid chromatography–mass spectrometry metabolomics was used to quantify selected serum metabolites involved in amino acid and related metabolic pathways. This analysis focused on acute immunometabolic disturbances during severe COVID-19 and their changes during recovery. Second, nuclear magnetic resonance-based plasma metabolomics was applied to broaden metabolic profiling by measuring low-molecular-weight metabolites, lipids, lipoprotein subclasses, and glycoprotein-associated inflammatory markers across three timepoints. Third, a larger multi-omics study integrated whole-blood RNA sequencing, targeted urine LC-MS metabolomics, urinary kidney injury biomarker profiling, clinical data, and electronic health record information. These studies are summarized in Table 1.

The analytical strategy evolved across the three studies. Initially, paired longitudinal comparisons were used to identify metabolites that changed between acute disease and early recovery within the same individuals. Case-control comparisons were used to assess whether acute and recovery-phase metabolomic profiles differed from population controls. In the NMR study,

the design was extended to three timepoints and included exploratory subgroup analyses. In the final multi-omics study, electronic health record data were used to stratify hospitalized patients into Long COVID and Recovered groups based on new post-acute diagnoses recorded during the 12 months after the acute disease period. This enabled analysis of both shared recovery trajectories and molecular differences associated with post-acute complications.

Statistical analyses included longitudinal comparisons between timepoints, group comparisons between patient subgroups and controls, pathway enrichment analysis, and integrative interpretation of transcriptomic, metabolomic, kidney biomarker, and clinical data. Multiple testing correction was applied where appropriate. The overall analytical goal was to distinguish acute infection-related molecular responses from persistent post-acute alterations and to identify biological pathways and clinically measurable markers associated with Long COVID risk.

Table 1. Summary of study designs and experimental approaches included in the thesis.

	Methods	Design and comparisons	Main purpose
1. Acute immunometabolic profiling	Serum targeted LC-MS metabolomics	Acute phase vs one-month recovery; comparison with population controls	To identify acute amino acid metabolism alterations and early recovery changes
2. Metabolic recovery profiling	Plasma NMR metabolomics; metabolites, lipids, lipoproteins, glycoprotein markers	Acute phase, one-month recovery, three- to four-month recovery; comparison with controls	To characterize broader metabolic recovery and persistent metabolic deviations
3. Longitudinal multi-omics	Whole-blood RNA sequencing, urine LC-MS metabolomics, urinary kidney injury biomarkers, clinical and EHR data	Three longitudinal timepoints; Long COVID vs Recovered stratification based on 12-month EHR data	To identify recovery trajectories and molecular signatures associated with post-acute complications

RESULTS

I Acute severe COVID-19 is characterized by amino acid metabolism dysregulation

The first study included a longitudinal targeted LC-MS metabolomics analysis of serum samples from hospitalized patients with severe COVID-19. Samples collected during the acute phase at hospital admission were compared with paired recovery samples collected approximately one month later and with population controls. This design allowed disease-stage-related metabolic alterations to be identified while reducing the influence of high inter-individual variability.

Targeted metabolomics revealed a distinct serum metabolomic profile during acute severe COVID-19. Principal component and clustering analyses showed that acute-phase samples differed from recovery-phase samples and population controls, indicating strong metabolic remodeling during hospitalization (Figure 1A,B). The most prominent changes involved amino acid metabolism, with pathway analysis highlighting tryptophan-, arginine-, glutamine/glutamate-, and phenylalanine/tyrosine-related pathways (Figure 1C,D).

The tryptophan-kynurenine pathway was one of the most affected metabolic routes. Tryptophan, kynurenine, and 3-hydroxy-DL-kynurenine differed significantly between acute and recovery phases, indicating increased tryptophan catabolism during acute severe disease. This pathway is closely linked to inflammatory and interferon-related immune activation and may contribute to immune regulation during SARS-CoV-2 infection.

Arginine-related metabolism was also altered. Significant changes in citrulline and ornithine suggested disturbances in arginine metabolism and urea-cycle-associated pathways. These metabolic alterations are relevant because arginine metabolism is connected with immune regulation, nitric oxide biology, endothelial function, and organ stress. Glutamine depletion was another key finding. Because glutamine supports immune cell proliferation, macrophage function, and cellular stress responses, reduced glutamine levels during acute disease were consistent with increased metabolic demand in severe infection.

Overall, the first study demonstrated that severe COVID-19 at hospital admission is characterized by coordinated amino acid metabolism dysregulation, especially involving tryptophan-kynurenine metabolism, arginine-related metabolism, glutamine depletion, and phenylalanine/tyrosine-related pathways. These findings established the first major thesis result: acute severe COVID-19 is not only a respiratory disease, but a systemic immunometabolic condition.

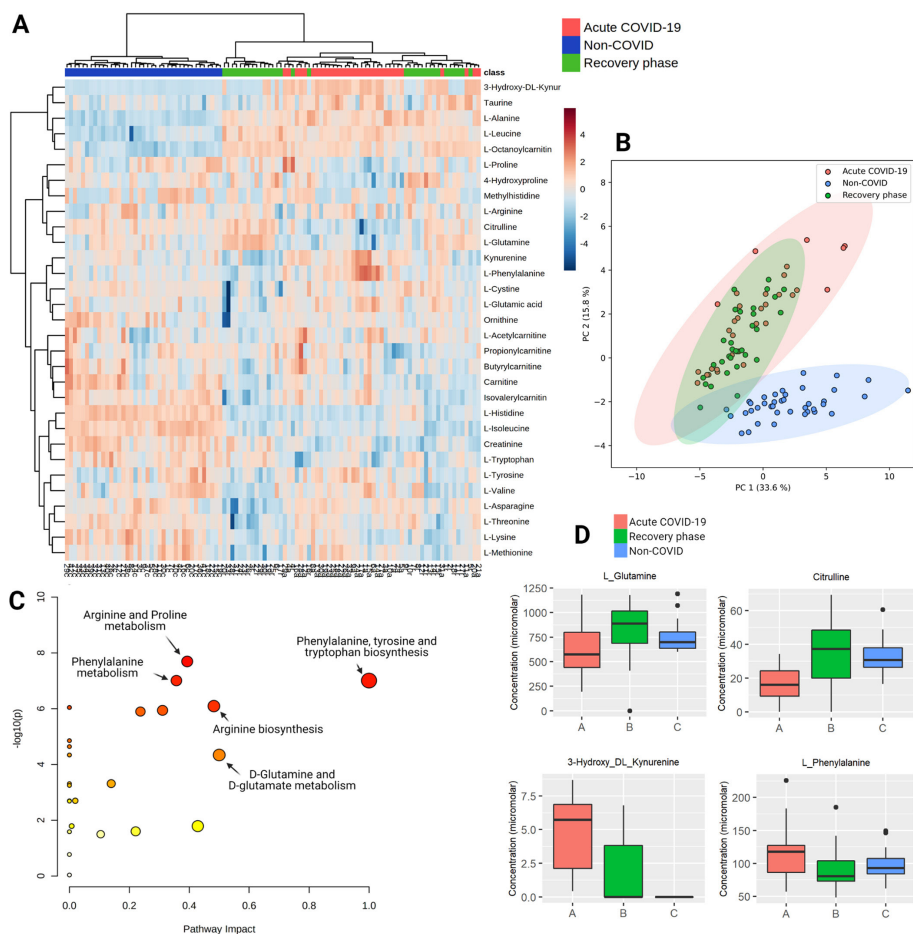


Figure 1. Targeted metabolomic analysis of longitudinal serum samples from hospitalized COVID-19 patients. (A) Heatmap and hierarchical clustering of the top 22 significantly altered metabolites in acute-phase, recovery-phase, and control samples. (B) Principal component analysis showing separation of acute COVID-19, recovery, and control metabolomic profiles. (C) KEGG pathway analysis showing the most affected metabolic pathways according to pathway significance and impact. (D) Boxplots of selected functionally relevant metabolites altered during recovery. Red: acute phase; green: recovery phase; blue: controls.

II Metabolic recovery after hospitalization remains incomplete in a subset of patients

The second study extended the targeted LC-MS findings by applying NMR-based plasma metabolomics across three timepoints: acute disease, one-month recovery, and three- to four-month recovery. This enabled broader profiling of low-molecular-weight metabolites, lipids, lipoprotein subclasses, and glycoprotein-associated inflammatory markers in hospitalized COVID-19 patients and population controls (Figure 2A,B).

Longitudinal NMR metabolomics confirmed that amino acid metabolism remained part of the recovery trajectory after severe COVID-19. Several amino acids, including histidine, methionine, phenylalanine, threonine, glutamine, tyrosine, and ornithine, contributed to time-dependent metabolic profiles. Although many amino acid concentrations tended to approach control levels by three to four months after hospitalization, recovery-phase samples remained distinct from population controls in some patients. This was supported by altered V5FC and ornithine levels and pathway-level changes involving nitrogen and amino acid metabolism (Figure 2 D,E). NMR profiling also revealed persistent dyslipidemia after hospitalization. Apo-A1 and HDL-cholesterol were altered during recovery and remained lower than in population controls in some patients. Additional changes in lipoprotein and triglyceride fractions indicated prolonged disruption of lipid transport and cardiometabolic homeostasis.

Glycoprotein-associated inflammatory markers were among the most consistent longitudinally altered features. GlycA, GlycB, and total Glyc were highest during acute hospitalization and decreased across recovery timepoints (Figure 2C). However, these markers remained elevated compared with population controls in many patients, supporting persistent low-grade inflammation during post-acute recovery.

Energy metabolism was also disturbed, with pathway-level analysis indicating persistent differences in glycolysis/gluconeogenesis, pyruvate metabolism, ketone body metabolism, and TCA-cycle-related pathways. Together, the NMR study showed that recovery after severe COVID-19 involves incomplete normalization of amino acid metabolism, alongside persistent lipid, inflammatory glycoprotein, and energy metabolism disturbances.

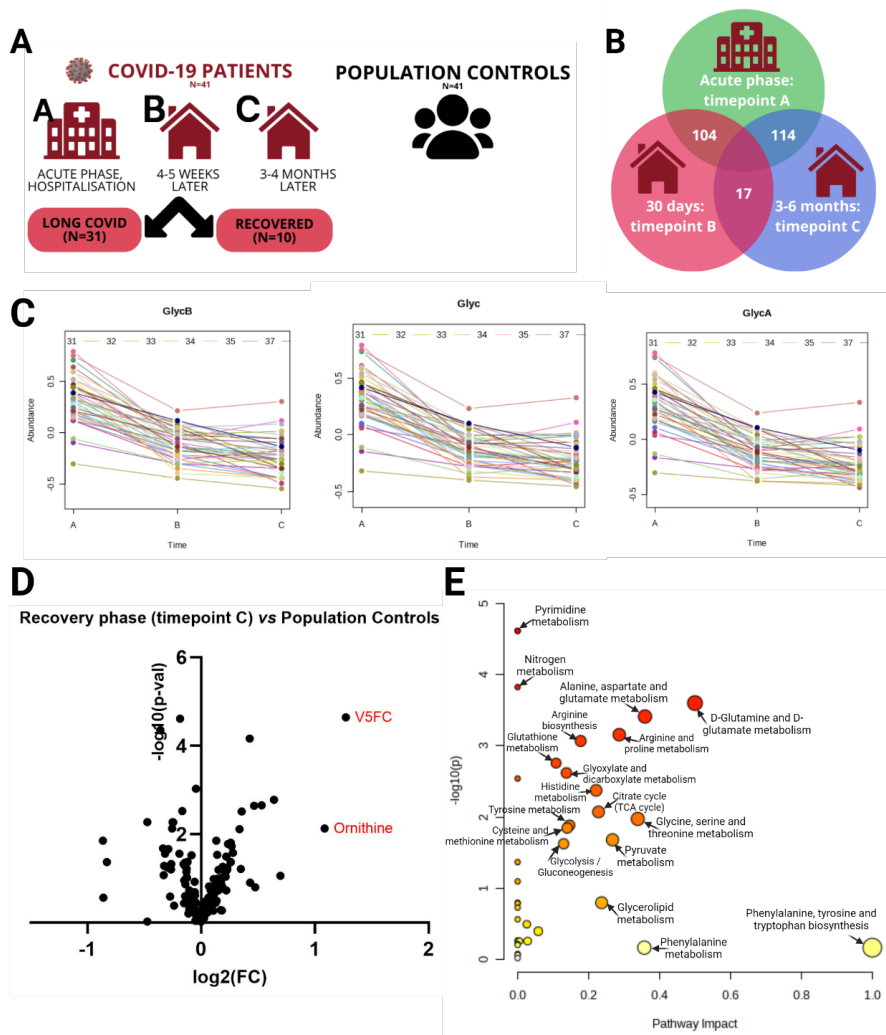


Figure 2. Longitudinal serum NMR metabolomics during acute and recovery phases after severe COVID-19. (A) Study design showing sampling during acute hospitalization, early recovery, and later recovery, with comparison to population controls. (B) Overlap of significantly altered metabolic features across the three timepoints. (C) Longitudinal changes in glycoprotein acetylation signals GlycA, GlycB, and total Glyc. (D) Volcano plot comparing recovery-phase samples with population controls, highlighting metabolites that remained altered after the acute disease phase, including V5FC and ornithine. (E) Pathway analysis of recovery-phase differences compared with population controls, indicating persistent involvement of nitrogen and amino acid-related metabolic pathways.

III Multi-omics signatures of immunothrombosis and Long COVID

The third study integrated whole-blood transcriptomics, targeted urine metabolomics, urinary kidney injury biomarker profiling, clinical measurements, and electronic health registry data to characterize molecular recovery after severe COVID-19. Hospitalized patients were followed longitudinally across three timepoints: acute disease at hospital admission, one-month recovery, and three-month recovery. Electronic health registry data were used to stratify patients into Long COVID and Recovered groups based on new post-acute sequelae of COVID-19 diagnoses recorded during the 12 months after acute infection (Figure 3A). In total, the multi-omics study included whole-blood transcriptomics from 81 hospitalized patients, and targeted urine metabolomics and kidney injury biomarker profiling in a subset of 61 patients.

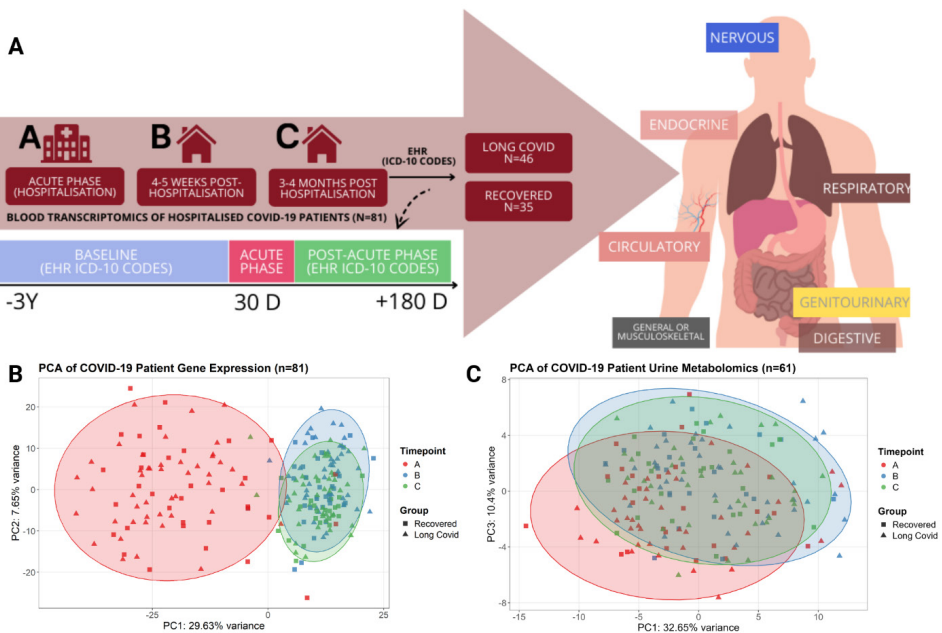


Figure 3. Longitudinal multi-omics study design and global omics structure in hospitalized COVID-19 patients. (A) Study design showing longitudinal sampling during acute hospitalization, one-month recovery, and three-month recovery, together with EHR-based classification into Long COVID and Recovered groups. (B) Principal component analysis of whole-blood transcriptomes showing separation of acute-phase samples from recovery-phase samples. (C) Principal component analysis of targeted urine metabolomics data showing timepoint-associated metabolic remodeling.

Across both transcriptomic and urine metabolomic datasets, the largest source of variation was the disease timepoint rather than the post-acute outcome group. Acute-phase samples separated clearly from recovery-phase samples in whole-blood transcriptomics, while urine metabolomics also showed timepoint-associated remodeling (Figure 3B,C). This indicated that severe COVID-19 induces a strong common molecular response during hospitalization and that recovery-related changes dominate the global omics structure. Therefore, the analysis first focused on longitudinal molecular remodeling across all hospitalized patients before examining Long COVID-specific differences. The acute-to-recovery transition was characterized by extensive whole-blood transcriptomic remodeling. More than ten thousand genes differed between acute disease and one-month recovery, while several hundred genes remained significantly altered between one- and three-month recovery, indicating that molecular recovery continued beyond hospital discharge and was not complete by one month. The largest transcriptomic changes were observed between hospitalization and one-month recovery, with genes involved in immune activation, inflammatory responses, mitochondrial function, and immunothrombotic processes among the most strongly altered (Figure 4A).

A major feature of acute severe COVID-19 was mitochondrial and bioenergetic dysregulation. Blood transcriptomic profiles showed activation of hypoxia-associated signaling, including HIF1A-related responses, together with changes consistent with increased glycolytic activity and reduced oxidative phosphorylation. This pattern suggested a shift toward glycolytic energy metabolism during the acute phase. In parallel, urine metabolomics showed elevated acylcarnitines during acute disease, supporting impaired mitochondrial β -oxidation and disrupted fatty acid metabolism. During recovery, genes involved in mitophagy, mitochondrial dynamics, oxidative metabolism, and heme biosynthesis showed rebound-like expression trajectories, including PINK1, OPA1, and FECH (Figure 4B–D). Together, these results supported a model in which severe SARS-CoV-2 infection induces acute mitochondrial dysfunction and energy metabolism disturbance, followed by gradual but still detectable mitochondrial repair processes up to three months after hospitalization.

The acute phase was also dominated by interconnected immunothrombotic pathways. Whole-blood transcriptomics showed activation of complement-related genes, neutrophil activation markers, neutrophil extracellular trap formation, platelet-related transcripts, and coagulation-associated genes. These changes supported a model in which complement activation, neutrophil extracellular trap formation, platelet activation, and coagulation form a self-amplifying immunothrombotic network during severe COVID-19. This acute immunothrombotic response provides a plausible mechanistic link between severe infection, vascular injury, and later post-acute complications.

stress during severe COVID-19 was connected to broader systemic metabolic disturbance rather than representing an isolated organ-specific process.

The most important phenotype-specific finding emerged at the three-month recovery timepoint. Patients who developed Long COVID showed a distinct blood transcriptional signature compared with recovered patients, whereas earlier timepoints did not show significant phenotype separation after multiple-testing correction. This three-month signature was characterized by increased expression of endothelial-associated, platelet-related, complement-regulatory, inflammatory, and tissue remodeling genes. Selected markers, including PROS1, VWF, CXCL5, FSTL1, ALOX12, and CFH, were higher in Long COVID patients than in recovered patients (Figure 5A).

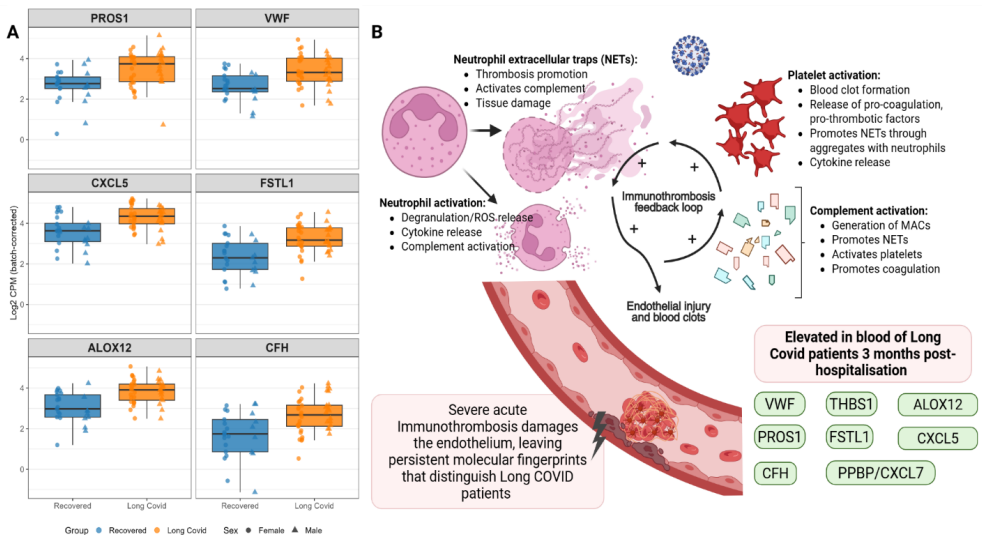


Figure 5. Persistent endothelial-associated and immunothrombotic signatures in Long COVID after hospitalization. (A) Expression of selected candidate genes that were increased in Long COVID patients compared with recovered patients at three-month recovery, including PROS1, VWF, CXCL5, FSTL1, ALOX12, and CFH. (B) Proposed model linking acute immunothrombosis with persistent endothelial-associated activation, platelet reactivity, complement regulation, and low-grade inflammation in Long COVID. The model summarizes interactions between neutrophil activation, neutrophil extracellular trap formation, platelet activation, complement activation, endothelial injury, and blood clot formation, highlighting molecular markers elevated in blood of Long COVID patients three months after hospitalization.

Increased VWF and PROS1 suggested persistent vascular perturbation and altered coagulation balance, while platelet-related markers supported ongoing platelet priming and prothrombotic activity. Increased CXCL5 indicated

persistent neutrophil recruitment and low-grade inflammatory signaling, whereas ALOX12 suggested lipid-mediated inflammatory activity and platelet-associated vascular inflammation. CFH upregulation may reflect compensatory regulation of ongoing complement activation. In addition, increased FSTL1 and other tissue remodeling-related genes suggested incomplete resolution of vascular or interstitial injury after severe infection.

Together, the multi-omics study identified immunothrombosis as a central molecular mechanism linking severe acute COVID-19 with post-acute complications. The results suggest that severe SARS-CoV-2 infection induces acute complement activation, neutrophil extracellular trap formation, platelet activation, mitochondrial dysfunction, and renal tubular stress. Although many of these pathways partially normalize during recovery, patients who develop Long COVID retain a persistent three-month blood transcriptional signature consistent with endothelial-associated activation, platelet reactivity, complement regulation, low-grade inflammation, and tissue remodeling (Figure 5B). These findings support the concept that severe COVID-19 can leave a durable vascular and immunothrombotic molecular imprint and identify clinically measurable candidate markers, including VWF, PROS1, complement-related genes, inflammatory mediators, and kidney injury biomarkers, for future post-acute risk stratification.

DISCUSSION

This doctoral thesis characterizes severe COVID-19 and post-acute recovery using longitudinal multi-omics profiling. The main finding is that severe COVID-19 is not limited to acute respiratory infection, but represents a systemic immunometabolic and vascular disease with persistent molecular consequences in a subset of hospitalized patients. Across the three studies, acute disease was characterized by amino acid metabolism dysregulation, inflammatory and glycoprotein-associated metabolic changes, lipid and energy metabolism disturbances, mitochondrial stress, renal tubular injury signals, and activation of immunothrombotic pathways. During recovery, many acute-phase alterations partially normalized; however, patients who later developed Long COVID retained a distinct three-month blood transcriptional signature consistent with endothelial-associated activation, platelet reactivity, complement regulation, low-grade inflammation, and tissue remodeling.

The first study demonstrated that amino acid metabolism is strongly altered during acute severe COVID-19. The most prominent changes involved the tryptophan-kynurenine pathway, arginine-related metabolism, glutamine depletion, and phenylalanine/tyrosine-related pathways. These findings are consistent with previous evidence that severe SARS-CoV-2 infection induces systemic immunometabolic reprogramming, where metabolites are not passive disease markers but are linked to immune activation, inflammatory signaling, and cellular energy demand (Ansone et al., 2021). Tryptophan catabolism through the kynurenine pathway is particularly relevant because it is induced by inflammatory and interferon-related responses and connects immune regulation with redox balance and NAD⁺ metabolism (O'Neill et al., 2016; Xu et al., 2024). Altered arginine-related metabolism may also be important because arginine, citrulline, and ornithine are linked with nitric oxide biology, endothelial function, and immune cell activity (O'Neill et al., 2016; Xu et al., 2024). Thus, the LC-MS study established acute severe COVID-19 as a condition of coordinated amino acid metabolism disruption rather than isolated metabolite changes.

The second study extended these findings by showing that metabolic recovery after severe COVID-19 remains incomplete in some patients. NMR metabolomics confirmed that amino acid-related changes were part of the recovery trajectory, but also revealed broader disturbances in lipid, lipoprotein, inflammatory glycoprotein, and energy metabolism. Persistent HDL- and Apo-A1-related alterations may be biologically relevant because HDL contributes to endothelial protection, reverse cholesterol transport, and anti-inflammatory functions.

GlycA, GlycB, and total Glyc were among the most consistent longitudinally altered markers, decreasing after the acute phase but remaining elevated in many patients compared with population controls. This supports the presence of persistent low-grade inflammatory activity after hospitalization. Together with pathway-level changes in glycolysis/gluconeogenesis, pyruvate metabolism, ketone body metabolism, and TCA-cycle-related pathways, these findings suggest that recovery from severe COVID-19 involves prolonged cardiometabolic and bioenergetic remodeling (Ansone et al., 2024).

The final multi-omics study provided a mechanistic framework linking acute severe infection with post-acute complications. Whole-blood transcriptomics showed that the largest molecular shift occurred between acute hospitalization and one-month recovery, indicating that acute disease produces extensive systemic remodeling. However, hundreds of genes remained significantly altered between one- and three-month recovery, showing that molecular recovery continues after hospital discharge. The major biological processes included immune activation, mitochondrial and energy metabolism changes, complement activity, neutrophil activation, platelet-related signaling, and coagulation. These findings support the concept that longitudinal omics is essential for separating transient acute responses from persistent recovery-related alterations.

A central finding of the multi-omics study was the identification of immunothrombosis as a dominant molecular axis. Immunothrombosis describes the interaction between innate immune activation and coagulation, involving complement activation, neutrophil extracellular trap formation, platelet activation, endothelial injury, and thrombus formation. This mechanism has been proposed as a key driver of acute COVID-19 pathology, particularly in hospitalized patients with systemic inflammation and vascular complications (Bonaventura et al., 2021). In this thesis, transcriptomic evidence of complement activation, neutrophil activation, NET-related genes, platelet-related transcripts, and coagulation-associated markers supports the presence of an interconnected immunothrombotic network during acute severe disease. Importantly, the persistence of endothelial-associated and platelet-related transcriptional features in Long COVID patients at three months suggests that acute immunothrombosis may leave a durable molecular imprint after the acute infection has resolved.

The three-month Long COVID signature was characterized by increased expression of VWF, PROS1, CXCL5, ALOX12, CFH, FSTL1, and other genes linked with vascular activation, platelet reactivity, complement regulation, inflammation, and tissue remodeling. These findings are consistent with broader evidence that Long COVID may involve persistent vascular dysfunction and thromboinflammatory processes. Recent studies have reported persistent complement dysregulation and thromboinflammatory signatures in active Long COVID, supporting complement and coagulation pathways as relevant

post-acute mechanisms (Cervia-Hasler et al., 2024). The observed upregulation of VWF and platelet-associated transcripts in this thesis is also consistent with the concept of ongoing endothelial-associated activation and platelet priming. However, because the transcriptomic data were obtained from bulk whole blood, these findings should be interpreted as blood transcriptional signatures of vascular and platelet-associated processes rather than direct measurement of endothelial tissue activity.

Mitochondrial and energy metabolism dysregulation represented another major theme. Acute severe COVID-19 was associated with hypoxia-responsive transcriptional patterns, increased glycolysis-related signaling, reduced oxidative phosphorylation components, and elevated urinary acylcarnitines, suggesting impaired mitochondrial β -oxidation. These results are compatible with a shift toward glycolytic metabolism during acute inflammatory stress. The recovery phase showed rebound-like expression of genes involved in mitophagy, mitochondrial dynamics, oxidative metabolism, and heme biosynthesis, including PINK1, OPA1, and FECH. This suggests that mitochondrial repair and metabolic reactivation remain active for months after hospitalization. Experimental evidence that SARS-CoV-2 proteins can directly affect mitochondrial function provides further biological plausibility for persistent mitochondrial stress after infection (Zong et al., 2023). Such mitochondrial and bioenergetic alterations may contribute to post-acute symptoms, although symptom-level causality could not be directly tested in this study.

The urine biomarker and metabolomics analyses showed that severe COVID-19 was also associated with renal tubular stress. Several kidney injury biomarkers were elevated during acute disease and decreased during recovery, suggesting partial renal recovery. Acute kidney injury is a recognized complication of severe COVID-19 and has been associated with adverse outcomes in hospitalized patients (Nadim et al., 2020; Sharma et al., 2021). In this thesis, kidney injury biomarkers correlated with metabolic features, supporting the interpretation that renal stress is connected to broader systemic immunometabolic disturbance rather than representing an isolated organ-specific phenomenon.

Several limitations should be considered. The Long COVID classification was based on electronic health registry diagnoses, which enables scalable follow-up but may be influenced by healthcare-seeking behavior, coding practices, and incomplete documentation. The cohort consisted of hospitalized patients with comorbidities and heterogeneous clinical trajectories, which may reduce power to detect phenotype-specific molecular differences. Long COVID itself is clinically heterogeneous, and distinct symptom subtypes may have different molecular mechanisms. Finally, bulk blood transcriptomics and targeted metabolomics provide systemic molecular information but cannot fully resolve cell-type-specific or tissue-specific processes.

Despite these limitations, the thesis provides an integrated longitudinal view of severe COVID-19 and recovery. The results support a model in which severe SARS-CoV-2 infection induces acute immunometabolic disruption, mitochondrial stress, renal tubular injury, and immunothrombosis. In many patients these processes partially normalize, but in a subset they leave persistent vascular, platelet, complement, inflammatory, and tissue remodeling signatures. These findings identify candidate clinically measurable markers, including VWF, PROS1, inflammatory glycoproteins, complement-related markers, lipid parameters, and kidney injury biomarkers, that may support future post-acute risk stratification and monitoring after severe COVID-19.

CONCLUSIONS

1. Severe COVID-19 is characterized by systemic immunometabolic dysregulation during the acute phase, with prominent alterations in amino acid metabolism, including tryptophan–kynurenine metabolism, arginine-related metabolism, glutamine depletion, and phenylalanine/tyrosine-related pathways.
2. Metabolic recovery after hospitalization is incomplete in a subset of patients. NMR-based metabolomics revealed persistent disturbances in lipid and lipoprotein metabolism, inflammatory glycoprotein markers, amino acid metabolism, and energy metabolism up to three to four months after severe COVID-19.
3. Longitudinal multi-omics profiling showed that recovery from severe COVID-19 involves extensive molecular remodeling. The largest transcriptomic changes occurred between acute hospitalization and one-month recovery, but significant molecular alterations persisted between one and three months after hospitalization.
4. Acute severe COVID-19 was associated with mitochondrial and bioenergetic dysregulation, including hypoxia-associated transcriptional responses, altered glycolytic and oxidative phosphorylation-related pathways, impaired fatty acid oxidation, and recovery-associated changes in mitophagy, mitochondrial dynamics, and heme metabolism.
5. Severe COVID-19 induced renal tubular stress, reflected by increased urinary kidney injury biomarkers and recovery-associated urine metabolite changes. These findings suggest that renal injury signals are connected with broader systemic metabolic and inflammatory disturbances.
6. Immunothrombosis emerged as a central molecular mechanism linking severe acute COVID-19 with post-acute complications. Complement activation, neutrophil activation and extracellular trap formation, platelet activation, coagulation-related signaling, and endothelial-associated activation formed an interconnected molecular network during severe disease.
7. Patients who developed Long COVID after hospitalization retained a distinct three-month blood transcriptional signature characterized by endothelial-associated activation, platelet reactivity, complement regulation, low-grade inflammation, and tissue remodeling.
8. The thesis identifies candidate markers for future post-acute risk stratification and monitoring after severe COVID-19, including VWF, PROS1, complement-related markers, inflammatory glycoproteins, lipid parameters, and urinary kidney injury biomarkers.

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