15

Untargeted Metabolomics and Lipidomics in COVID-19 Patient Plasma Reveals Severity Biomarkers

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Abstract

Introduction: Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, has widely varying clinical severity. Currently, some biomarkers used to assess severity of COVID-19 patients include interleukin-6 (IL-6) and C-reactive protein (CRP), which are inflammatory markers and not specific to COVID-19 disease. The goal of this study is to gain mechanistic insights at the molecular level by integrating LC/MS-based metabolomics with clinical data to understand the differences in severity of the infection, and to discover predictive biomarkers related to the outcomes among the COVID-19 patients.

Methods: This cohort study (n=76) included patients aged 16-78 years who tested positive for SARS-CoV-2 and were enrolled from inpatient hospitals and outpatient testing centers in Memphis, TN between August 2020-July 2022. The protocol was approved by the UTHSC and the FDA Research Involving Human Subjects Committee. Untargeted metabolomics analysis was conducted to discover metabolites that correlated with COVID-19 severity.

Preliminary Data: Plasma IL-6 levels measured using the OLINK platform had significant increases in Severe vs Mild group. The metabolomics data showed that the tryptophan pathway was altered in severe COVID-19 patients; specifically, increases in kynurenine and decreases in cyclic melatonin, which are involved in regulation of inflammation and immunity were noted in patients with severe disease. Additionally, higher levels of glucose were found in severe COVID-19 patients and might be caused by hemolysis. Correlation analysis demonstrated that IL-6 (inflammation biomarkers) had strong positive correlations to ceramides and 4-hydroxybutyric acid, with negative correlations to LPCs. In summary, metabolomics analysis of plasma samples identified potential biomarkers that correlate with severity of COVID-19 disease.

Experimental Design

SARS-CoV-2 (+)
Mild Patients (n = 39)
Severe Patients (n = 37)

Mild or Severe classification was based on cumulative scores of ≤3 and ≥4, respectively

Blood plasma
On the day of enrollment

Metabolomics & lipidomics analysis
Using UHPLC/HRMS

- **Raw Data analysis** Using Compound Discovery & LipidSearch
- **Multivariant analysis**
- **Correlation analysis**

Scoring criteria:

i) **2 points for hospital admission within 60 days of enrollment;**

ii) **3 points for ICU admission within 60 days of enrollment;**

iii) **1 point for mild symptoms: cough, fever, diarrhea, vomiting, headache, loss of taste or smell, sore throat, myalgias, fatigue, lymphadenopathy, and malaise;**

iv) **2 points for symptoms: shortness of breath (dyspnea), wheezing, SpO2<92% on room air, respiratory rate (RR)>30, and new non-invasive oxygen requirement.**

v) **2 points for symptoms: invasive or positive-pressure oxygen requirement, acute kidney injury (Cr >1.5x upper limit normal for age or estimated glomerular filtration rate [eGFR] <60), elevated aspartate/alanine transaminase ((AST/ALT); ratio >2x normal), new elevation international normalized ratio (INR) >1.3, and altered mental status;**

vi) **3 points for symptoms: acute respiratory distress syndrome (ARDS), shock requiring pressors, renal failure with dialysis, extracorporeal membrane oxygenation (ECMO) requirement, organ transplant, pulmonary embolism, deep venous thrombosis, and/or stroke.**

Results

Table 1. Patient demographics by severity

	Severity		
	Mild	**Severe**	**p**
N	39	37	–
Scoring	1.17 ± 0.72	7.35 ± 2.92	1.9E-15
Days from first diagnostic to plasma collection	6.12 ± 4.66	10.2 ± 13.8	0.096
Age	41.8 ± 13.6	52.8 ± 17.7	0.004
Black	6 (15.4%)	26 (70.3%)	0.002
White	18 (46.1%)	10 (27.0%)	0.084
Other Race	15 (38.5%)	1 (2.7%)	0.0001
Female	17 (43.6%)	19 (51.3%)	0.50
Underlying health conditions			
BMI	N/A	32.6 ± 7.16	-
Diabetic	4 (10.3%)	16 (43.2%)	0.002
Hypertension	8 (20.5%)	28 (75.7%)	0.0005
Cardiovascular diseases	4 (10.2%)	17 (45.9%)	0.002
Chronic renal disease	1 (2.6%)	7 (18.9%)	0.027
Severe obesity	5 (12.8%)	17 (45.9%)	0.002
Chronic lung disease	0	10 (27.0%)	0.0004
ICU sickness			
Sepsis	0	11 (29.7%)	0.0001
Pneumonia	0	23 (62.2%)	3.6E-10

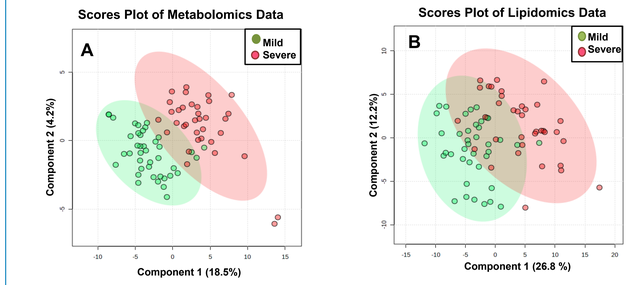

Figure 1. Partial least-squares discriminant analysis (PLS-DA) scores plots of metabolome data (**A**) and lipidome data (**B**) from Mild and Severe COVID-19 patients.

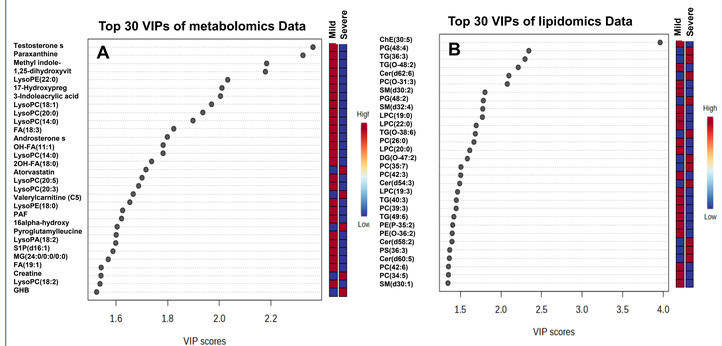

Figure 2. The top 30 variable importance in projection (VIP) plot of metabolites **(A)** and lipids **(B)**, which contributed significantly to group separation.

Results

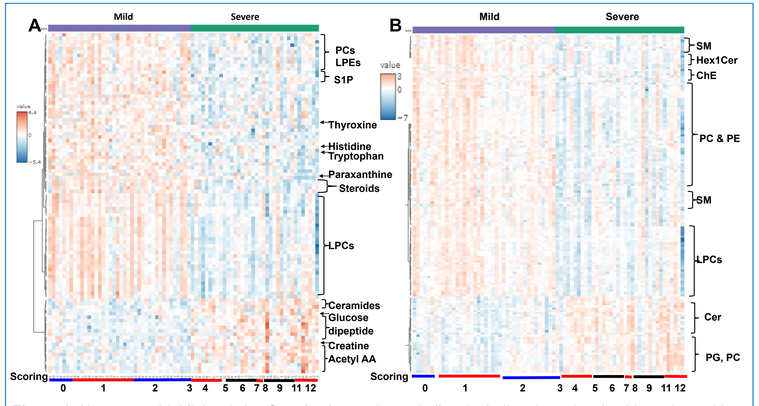

Figure 3. Heat maps highlighted significantly changed metabolites including those involved in amino acid (AA) metabolism, purines, acylcarnitines, fatty acids, ceramides, PCs and lysoPCs **(A)**, and significantly changed lipids including ceramides, PCs and lysoPCs **(B)**. *Each column represents data from each individual patient with total_Severity_Score displayed at the bottom of the heat map plot.*

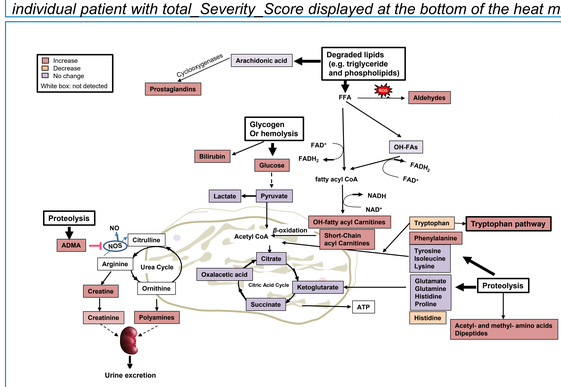

Figure 4. Summary illustration of the identified changed-metabolites involved in AA metabolism, urea cycle and energy pathways in sera from Severe COVID-19 patients vs Mild.

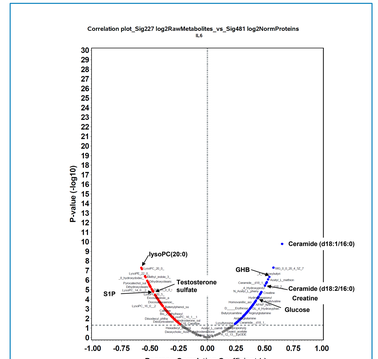

Figure 5. Correlation plot of metabolites to IL6 (inflammation biomarker), which was measured using OLINK. Note: the labeled metabolites are the common metabolites with significant strong correlations to severity scoring, IL6 and to creatine (a renal function biomarker).

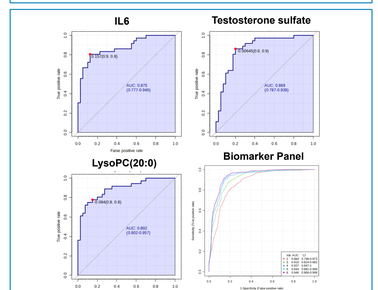

Figure 6. ROC curves of IL6, testosterone sulfate, lysoPC(20:0) and a biomarker panel containing 6 metabolites with AUC of 0.875, 0.869, 0.892 and 0.946, respectively. From the top correlation data, the biomarker panel include testosterone sulfate, S1P (d16:1), glucose, LPC(20:0), 4-hydroxybutyric acid (GHB), and ceramide(d18:1/16:0).

Conclusion

- Analysis of demographic and comorbidity prevalence showed age (p = 0.004), race (p = 0.002), diabetes (p = 0.002) and hypertension (p = 0.0004) are risk factors for Severe COVID-19 patients.
- Increases in kynurenine and decreases in cyclic melatonin indicated that tryptophan pathway was disturbed more in Severe vs Mild patients. Hyperactivation of the kynurenine pathway might cause an increased susceptibility to infection by immunosuppressive activity.
- Higher levels of proteolysis amino acids including acetyl-amino acids and dipeptides observed in Severe patients might be the result of muscle protein catabolism to provide necessary amino acids to compensate for insufficient dietary protein intake and depleted protein stores as well as to provide building blocks (AA) for rapid proliferation of virus to produce its required proteins.
- The consistent general findings of decreases in LPCs and PCs from both metabolomics and lipidomics data indicates that the phospholipids were hydrolyzed to building blocks to assemble virus membrane for virus proliferation.
- Based on correlation data to severity scoring, IL6 and to creatine, a biomarker panel, including testosterone sulfate, S1P (d16:1), glucose, LPC(20:0), 4-hydroxybutyric acid (GHB), and ceramide(d18:1/16:0), provides a better prediction with AUC=0.946 vs 0.875 from IL6.
- This study has limitations including no matched healthy subjects and limited number of patients. Although consistent with previous studies, the findings still warrants further investigation in a larger study.