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Trapped Ion Mobility PASEF Based Lipidomics Highlights Potential Lipid Biomarkers of Covid-19 severity

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Introduction

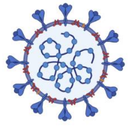
- **SARS-CoV-2** was first reported in late 2019 and to date more than 160 million people have contracted the virus and almost 4 millions confirmed deaths have been documented.
- **Covid-19 disease** presents a **varied symptomatology**: In most cases it appears with **mild** symptoms but can evolve into a **severe** condition with an acute respiratory syndrome requiring **hospitalization** and **intensive cares**.
- The identification of possible **prognostic Biomarkers**, which may predict the course of the disease or the response to therapeutic treatments, becomes a **fundamental goal**.
- Lipids play a key role in the regulation of numerous pathways, in this study a **4D-Lipidomics** approach was performed for the analysis of **Covid-19 plasma samples** of patients with different degree of severity.

Methods

- Lipids were extracted from plasma by the **Matyash[1] method (MeOH/MTBE/H_2O).**
- **RP-UHPLC-TIMS-MS/MS** was employed for untargeted lipidomics data acquisition.
- **PASEF** mode was used for DDA, in both ESI(+) and ESI(-) ionization.
- **MetaboScape 2021** and **MetaboAnalyst 5.0** were used for Identification and Statistical analysis.

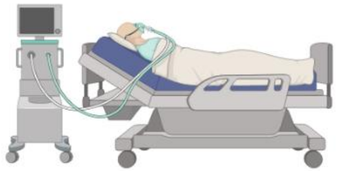
[1] Matyash V., et al. 2008, J. Lipid. Res., https://doi.org/10.1194/jlr.D700041-JLR200

Study Design



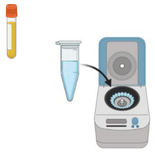
❑ **Enrollment**

- Patients were classified according to the clinical phenotype following World Health Organization (WHO) severity score as *Mild* (n= 44) patients or *Severe* (n= 54) patients.
- Healthy and SARS-CoV-2 negative subjects (n = 21) were recruited from healthcare workers.



❑ **Blood sample processing**

- From each patient, 5-8 mL of whole blood was drawn into EDTA vacutainers and centrifuged at 1,000 x *g* for 20 minutes at 25°C to separate blood cells and plasma. After collection, plasma samples were aliquoted and stored at – 80°C until analysis.



❑ **Plasma lipidome extraction**

- Lipids were extracted from 20 µl of plasma, spiked with a mix of deuterated standards (Splash Lipidomix®, Avanti Polar).
- A pooled QC was prepared to assess repeatability.

Analytical Workflow

❑ **Analytical platform**

- Untargeted lipid profiling was performed by RP-UHPLC hyphenated to a timsTOF Pro (Bruker) Q-TOF mass spectrometer.
- Lipids were separated on a CSH 100 mm x 2.1 mm, 1.7 µm column (Waters), using as mobile phases: A) 10mM $HCOONH_4$ + 0,1% HCOOH H_2O/ACN 60/40 (v/v) B) 10mM $HCOONH_4$ + 0,1% HCOOH IPA/ACN 90/10 (v/v). T=55°C.

❑ **Lipid annotation and statistical analysis**

- Identification was performed in **MetaboScape** (Bruker) by using LipidBlast spectral library and Rule-Based Annotation. MS/MS score, ΔCCS% values (vs predicted) and retention time linearity were considered for lipid annotation.
- **Pre-processing**: Internal standard normalization, log transformation, auto-scaling.
- **MetaboAnalyst**: Univariate (one-way ANOVA) and multivariate (PLS-DA) analysis were employed to identify statistically significant lipids and to visualize class separation. A Random Forest (RF) model was trained and tested to predict severity.

Statistical Analysis

Figure 1

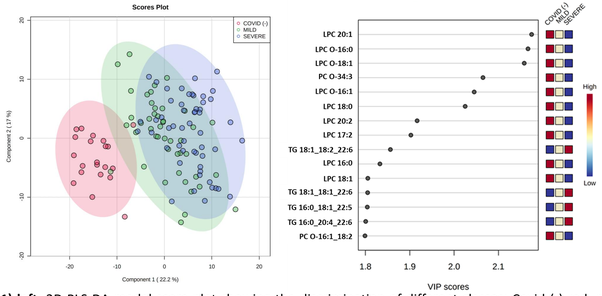


Figure 1) left: 2D PLS-DA model score plot showing the discrimination of different classes: Covid (-) red, mild: green, severe: blue; R^2 and Q^2 results estimated via cross validation and permutation test results based on 1000 iterations; **right:** The 15 highest scoring variable importance in projection (VIP) metabolites are shown. The number of VIPs was established by setting the VIP-score ≥ 1.8 as a cutoff value. The colored boxes on the right indicate the relative amount of the corresponding lipid compound in each group.

Figure 2

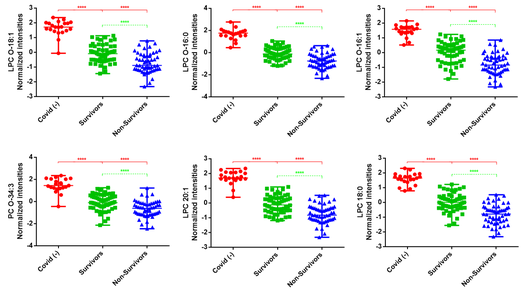


Figure 2) Comparison of normalized intensity (based on labelled internal standards mixture, log transformed and autoscaled dataset) of the selected lipid panels in mild and severe survivors compared to non-survivor patients, **** $p < 0{,}0001$

Figure 3

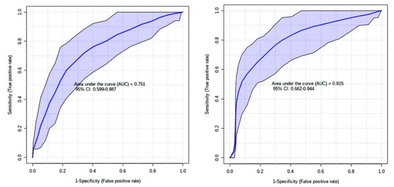


Figure 3) ROC curves for severity (left) and outcome (right) obtained with the predictive model (RF) on the reduced lipid panel composed by LPC O-18:1, PC O-34-3, LPC 20:1, LPC O-16-1, LPC 18:0, LPC O-16:0.

Results

- **348 lipids** were confidently annotated, belonging to different lipid classes. Avg. MS/MS score: 914.60, Δppm: 0.60, ΔCCS%: 1.30%, avg. CV% (rt and area) ≤ 12%.
- **PLS-DA** highlighted a distinct **class separation**, among 15 highest scoring VIP lipids **9 were LPCs**. 191 lipids were found significantly modulated ($p < 0.01$, FDR: 0.01%).
- **A progressive increase of TGs, Cer, PEs levels was found** from mild to severe patients, **whereas a marked decrease of SMs, HexCer and mostly LPCs, LPC-O and PC-O** was observed in severe patients.
- **A RF model was built** to evaluate the **predictive potential** of the lipidome signature.
- The model provided AUC values of 0.751 (95% CI: 0.599-0.887 for severity and 0.815 (95% CI: 0.662-0.944) for outcome. **Using a reduced panel of six lipids selected as the most significant, the model showed the same predictive ability.**

Discussion

- The target of our study was to identify, at the time of hospitalization, **a potential lipid signature** able **to predict the progression of Covid-19.**
- The **RP-UHPLC-TIMS-PASEF** approach resulted in **high lipidome coverage** and showed **high repeatability** across the batches.
- **LPC class was found as the most discriminant** across the different conditions. In addition, **non-survivor patients** (either mild and severe) **showed lower values** with respect to survivors.
- **A restricted panel of six lipids** composed by LPC O-18:1, LPC 20:1, LPC O-16-1, LPC 18:0, LPC O-16:0, PC O-34-3 showed high **predictive ability** in **discriminate severity and mortality.**
- These results could suggest the potential employment of the defined lipidome signature as **prognostic tool** in **targeted approaches** and to **evaluate the recovery** of patients after discharge from the hospital.

Appendix: *LPC (Lysophosphatidylcholine), LPC-O (Ether-linked lysophosphatidylcholine), PC (Phosphatidylcholine), PC-O (Ether-linked phosphatidylcholine), TG (Triglycerides), PE (Phosphoethanolamines),Cer (Ceramides), HexCer (Hexosyl Ceramides), SM (Sphingomyelins).*