

A comparative NMR-based metabolomics study of lung parenchyma of severe COVID-19 patients

Joaquín I. Hurtado

Laboratorio de Interacción Hospedero Patógeno - UBM. Institut Pasteur de Montevideo

Andrés López-Radcenco

Universidad de la República

José Luis Izquierdo García

Universidad Complutense de Madrid

Guillermo Moyna

Universidad de la República

Fernando Rodriguez

Hospital Español - ASSE

Gonzalo Greif

Laboratorio de Interacción Hospedero Patógeno - UBM. Institut Pasteur de Montevideo

Nicolas Nin (✉ niconin@hotmail.com)

Hospital Español - ASSE

Short Report

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Abstract

The COVID-19 pandemic was the leading cause of mortality due to a single infectious agent in 2020 and 2021. The fatality rate for individuals admitted to the ICU for this condition was over 60%. A metabolomic analysis based on nuclear magnetic resonance was performed on patients who died in the ICU due COVID-19 and other fatal respiratory diseases. Although there is information on metabolic signatures in COVID-19 patients' serum and plasma, little is known about the infection site in the lungs. We found statistically significant differences between metabolites related to energy metabolism and inflammatory processes, revealing a unique metabolic profile.

Introduction

The ongoing Coronavirus disease 2019 (COVID-19) could cause respiratory and systemic compromise such as pneumonias-like symptoms, acute respiratory distress syndrome (ARDS), and multiorgan failure¹. The majority of patients diagnosed with COVID-19 attended the disease at home, 5–6% needed hospitalization in moderate care and between 1–2% required admission in the Intensive Care Unit (ICU). In case series reported in the last year, hospital mortality was around 59% in patients admitted to the ICU, increasing up to 80% in those who required mechanical ventilation².

Comorbidities including hypertension, diabetes, cardiovascular disease, and age may be potential risk factors among patients admitted in ICU³. On the other hand, viral load and also dysbiosis of respiratory and gut microbiota could influence the clinical manifestations of COVID-19⁴. There is still no effective therapy for severe ill patients and a comprehensive understanding in metabolic changes and sepsis biomarkers are needed to address an improvement in the clinical management of patients.

Since it is now well accepted that COVID-19 is a systemic disease, several studies have employed metabolomic approaches to better understand the metabolic pathways involved in COVID-19 pathogenesis. Although there are many metabolomic studies in respiratory infections, the vast majority are focused on serum, plasma and bronchoalveolar lavage fluid samples. Recently, metabolomic studies in serum of patients with ARDS due to COVID-19 revealed an altered amino acids metabolism, lipid metabolism, glycolysis and anaplerotic metabolism, suggesting an alteration in energy pathways, inflammatory response and oxidative stress⁵.

In this work we used an NMR-based non-targeted metabolomics approach to characterize the metabolome of lung parenchyma from fatal COVID-19.

Methods

The study was approved by the Ethics Committee from “Hospital Español - ASSE”, a COVID-19 reference public hospital in Montevideo, Uruguay. Fragments of lung tissue were collected during clinical autopsy performed on patients with COVID-19 (n = 8) between November 2020 and February 2021 admitted in the

ICU. Confirmation of infection by SARS-CoV-2 was made by RT-qPCR during ICU stay and informed consent was signed by direct family members before autopsy. All samples were obtained in the first 2 h *post-mortem* and stored at -80°C.

On the other hand, lung fragments from non-COVID-19 deceased patients were collected between December 2016 and June 2018 also at Hospital Español – ASSE. This group includes microbiological and serological positive results for *Klebsiella pneumoniae*, *Leptospira interrogans*, and respiratory syncytial virus (n = 6). In all cases, lung tissues were obtained immediately after confirmation of death, and were stored immediately at -80°C for further studies.

The inclusion criteria for both groups were adults older than 18 years, admitted in the ICU with respiratory sepsis, septic shock or multi-organ failure, and all patients were mechanically ventilated.

An adaptation of previously published methods⁶ was performed and lung fragment samples were homogenized and extracted with CHCl₃/MeOH/H₂O. Briefly, lung tissue was homogenized in water and methanol in a Bullet Blender (Next Advance, USA). Subsequently, chloroform was added for the final ratio of 8:4:3 CHCl₃:MeOH:H₂O respectively, mixed in a vortex for 5 minutes, and centrifuged for 5 minutes at 5000g. Aqueous phase was lyophilized and resuspended in buffer phosphate in deuterium water (D₂O) pH 7.4. Water-suppressed 1D-NOESY ¹H NMR spectra of aqueous tissue extracts were obtained at 500 MHz in a Bruker AVANCE III 500. A spectral width of 10 KHz, a data size of 32 K, and a total of 128 scans were employed to record each spectrum, using a relaxation delay of 4 s between scans. When required, gradient enhanced heteronuclear single quantum correlation (HSQC) protocol and 1D total correlated spectroscopy (TOCSY) spectra were acquired using standard pulse sequences provided with the spectrometer.

Results

As indicated in Table 1, all the patients in this study had acute respiratory sepsis and had high APACHE-II scores upon admission (mean 20.6 ± 8.4 points). Patients required mechanical ventilation and were on vasopressor support in all cases. Multiorgan failure occurred in all of the COVID-19 patients, with an average ICU stay of 17.6 ± 4.9 days. When compared to non-COVID pneumonias, they also had a higher percentage of comorbidities on admission (diabetes, hypertension, COPD, or obesity) and a lower PAFI (PaO₂/FiO₂) score. The latter group spent an average of 11.2 ± 8.3 days in the ICU and had a slightly lower rate of multi-organ failure (83%).

Table 1

Demographic and clinical characteristics of the study population upon admission in UCI

	COVID-19 (n = 8)	Non-COVID-19 (n = 6)
Demographic features		
Age, mean (SD ^a)	68.6 ± 8.2	57.3 ± 17.1
Female, n (%)	3 (37)	3 (50)
APACHE-II score, mean (SD)	20.6 ± 8.4	19.2 ± 10.2
Comorbidities, n (%)		
COPD ^b	4 (50)	2 (33)
Diabetes	3 (38)	0 (0)
Hypertension	7 (88)	1 (17)
Obesity	3 (38)	0 (0)
General findings		
PAFI ^c day 1, mean, (SD)	115 ± 31	230 ± 162
ARDS ^d	3 (38)	1 (17)
Vasopressor support	8 (100)	5 (83)
Renal failure	5 (63)	2 (33)
Multiorgan failure	8 (100)	5 (83)
Mechanical ventilation, days, mean (SD)	16.4 ± 5.3	9.7 ± 7.1
ICU length of stay, mean (SD)	17.6 ± 4.9	11.2 ± 8.3
^a Standard deviation, ^b Chronic obstructive pulmonary disease, ^c The arterial oxygen pressure/inspired fraction of oxygen (PaO ₂ /FiO ₂ or PAFI) is a gas exchange indicator that has been widely used to assess the severity of lung damage in critically ill patients. ^d Acute respiratory distress syndrome		

As shown in Table 2, the analysis indicates different metabolomic profiles between both cohorts, showing an increase or decrease of some specific metabolites in the tissue samples.

We compared the NMR spectra of eight lung tissue extracts from COVID-19 autopsies, and six non-COVID-19 autopsies. Although the low number of samples of each condition, principal component analysis (PCA) showed good discrimination between groups [see Additional file 1]. In addition, statistical

differences between groups were observed. Orthogonal projections to latent structures discriminant analysis and statistical total correlation spectroscopy analyses of the resulting data, in combination with classical NMR dereplication experiments, allowed us to identify 21 metabolites among the two cohorts, 11 of them were differentially expressed (Table 2). The amino acids valine, alanine, methionine, glycine, tryptophane, phenylalanine, tyrosine, asparagine and the metabolic intermediate fumarate, were significantly increased in samples from COVID-19 patients. On the other hand, choline and α -glucose were significantly decreased among these samples.

Table 2

Comparison of metabolites between fatal COVID-19 and non-COVID-19 patients. p-value < 0.05 are indicated with bold numbers.

	<i>COVID-19 patients</i>		<i>Non-COVID-19 patients</i>		<i>Fold change</i>	<i>p-value</i>
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>		
<i>Valine</i>	1.480	0.677	0.723	0.246	-0.31	0.008
<i>Isoleucine</i>	1.508	2.527	0.400	0.132	-0.58	0.128
<i>Beta-hydroxybutyrate</i>	0.246	0.116	0.205	0.063	-0.08	0.217
<i>Lactate</i>	15.958	4.933	17.677	3.053	0.04	0.214
<i>Alanine</i>	1.842	0.205	1.306	0.304	-0.15	0.001
<i>Methionine</i>	0.212	0.116	0.109	0.038	-0.29	0.021
<i>Ceratine</i>	0.655	0.236	0.523	0.214	-0.10	0.139
<i>Cholin</i>	3.262	0.808	5.341	1.662	0.21	0.008
<i>P-choline</i>	1.050	0.342	1.010	0.250	-0.02	0.400
<i>Betaine</i>	0.395	0.330	0.330	0.211	-0.08	0.327
<i>Glycine</i>	1.645	0.229	1.202	0.207	-0.14	0.001
<i>α-Glucose</i>	0.066	0.022	0.225	0.210	0.54	0.046
<i>β-Glucose</i>	0.204	0.078	0.454	0.351	0.35	0.056
<i>Histidine</i>	0.129	0.123	0.040	0.014	-0.51	0.052
<i>Tryptophan</i>	0.054	0.026	0.028	0.003	-0.29	0.011
<i>Uracil</i>	0.074	0.018	0.059	0.022	-0.10	0.102
<i>Phenylalanine</i>	0.564	0.254	0.272	0.067	-0.32	0.007
<i>Tyrosine</i>	0.415	0.134	0.180	0.052	-0.36	0.001
<i>Fumarate</i>	0.027	0.012	0.009	0.003	-0.47	0.002
<i>Glutamate</i>	2.309	0.426	2.465	0.488	0.03	0.263
<i>Asparagine</i>	0.145	0.047	0.070	0.030	-0.32	0.002

Discussion

The aim of this study was to compare the metabolome of lung parenchyma in fatal COVID-19 from other severe infections and whether we could discriminate a distinctive metabolic profile using NMR-based

non-targeted metabolomics technique. We found good separation in PCA analysis which indicates a distinct metabolic footprint in lung parenchyma of COVID-19 infection.

In first place, lactate was the most widely expressed metabolite across cohorts with no statistically significant differences between them. This finding is consistent with the known fact that high plasma lactate concentrations are a marker of poor prognosis and an indicative of metabolic acidosis in critically ill patients and were expected to be higher in both groups⁷. In fatal COVID-19, we also found a significant increase in several tissue amino acids, such as phenylalanine, alanine, valine, methionine, and asparagine. In patients with COVID-19, amino acid metabolism may be altered as a compensatory mechanism to restore NAD reducing power levels in the context of lung injury and hypoxia⁸. Inflammatory markers like tryptophan⁹, on the other hand, are higher in COVID-19 than in other acute pneumonias. Lung injury in COVID-19 leading to severe refractory hypoxemia was observed in recently published series, and is one of the main causes of mortality in this study.

Choline levels were also found to be significantly lower in COVID-19 samples. This has been reported in previous studies in the serum of severe COVID-19 patients, where an increase in choline consumption caused by activation of macrophage TLRs receptors was linked to extracellular cytokine secretion¹¹. The presence of pro-inflammatory components in bronchoalveolar lavage fluid is elevated even in severe COVID-19 patients treated with glucocorticoids, suggesting that slowing down the cytokine storm is a critical strategy for disease control¹².

One of the main limitations of our study is the low number of cases, but we were able to find statistical differences in metabolite concentrations between groups. We also excluded healthy controls since we decided to compare COVID-19 biomarkers with severe non COVID-19 pneumonias.

In conclusion, distinct metabolic signatures including energy metabolism and inflammatory pathways have been identified that distinguish COVID-19 from other deadly pneumonias induced by multiple respiratory infections.

Declarations

Ethics approval and consent to participate

The study was approved by the hospital's ethical committee (Bioethics and Research Ethics Committee - Hospital Español) and carried out in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki's guiding principles. Direct family members or the patient's legally authorized representative provided informed consent if the patient was unable to consent for any reason.

Consent of publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during this investigation are not publicly available owing to individual privacy concerns, but they will be made available upon reasonable request from the corresponding author.

Competing interests

The authors declare that they have no competing interests

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Authors' contribution

JH and NN wrote the first draft. AL, JLI and GM analyzed the database. All authors contributed to the article and approved the submitted version

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GG, GM and NN are researchers from Sistema Nacional de Investigación (ANII, Uruguay).

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