

Abnormal concentration of porphyrins in serum from COVID-19 patients

COVID-19 is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 is not only a lung disease but rather a systemic syndrome where alterations in the blood may play a key role.¹ Severe cases show a marked variation in the red blood cell distribution width,² which agrees well with reduced erythrocyte turnover and would function as a compensatory mechanism to maintain the circulating red blood cell and oxygen levels.³ Clinical evaluation of patients revealed low haemoglobin levels along with increased total bilirubin and ferritin concentrations.⁴ Hyperbilirubinaemia is also observed in erythropoietic porphyrias^{5,6} or inflammation processes and would be consistent with ineffective erythropoiesis⁷ and rapid haemoglobin turnover. Autopsies of deceased COVID-19 patients have revealed that the spleen is significantly altered in size, which is a normal physiological response to anaemia. Preliminary studies suggest that CD147 can bind the spike protein of SARS-CoV-2, abrogating the normal recycling of erythrocytes from the spleen into the bloodstream, selectively trapping red blood cells in the spleen and developing a form of anaemia.⁸

Porphyrin accumulation can occur due to pathological conditions, including severe infections or in porphyrias, the latter being a set of metabolic disorders that result from the deficiency of the enzymes in the haem biosynthetic pathway or gain-of-function mutations in aminolevulinate synthase 2 (ALAS2).⁵ Haeme synthesis relies on the sequential action of eight enzymes, mainly expressed in liver and in erythroid cells (Fig 1A) and requires a porphobilinogen ring flip to generate the type III porphyrins, precursors of haem. Yet, type I porphyrins also form by spontaneous cyclisation of the unstable hydroxymethylbilane (HMB), without inversion of the ring configuration. Spontaneous or effector-induced chemical degradation likewise affects several intermediates in the haem pathway to generate a number of toxic by-products, including the oxidation of porphyrinogens to porphyrins.

Thus, a central question is whether COVID-19 patients present porphyrin accumulation and, if so, to what extent. Here we have addressed this open issue by quantifying the total porphyrin content in the sera of a cohort of 134 COVID-19 patients (COVID-pos, Table I) reported to be in the acute phase of the disease as samples were collected upon the patients' admission to the hospital and confirmed by positive polymerase chain reaction (PCR) testing. We also analysed a cohort of 60 PCR-negative patients (COVID-neg) but also undergoing pneumonia and 54 serum samples collected in

2018–2019 (i.e., well before start of the COVID-19 pandemic) during an annual medical check-up (pre-COVID).

Results and discussion

We determined serum porphyrin levels by high-performance liquid chromatography (HPLC) separation and quantification. While these measurements are more sensitive on blood, the current emergency situation precluded working with such samples. Yet, serum is also a perfect matrix to derive the porphyrin content and the results are largely comparable.⁹ As shown in Fig 1B, the by-products uroporphyrin I (URO I) and coproporphyrin I (COP I) and the metabolite coproporphyrin III (COP III) are significantly accumulated in the serum porphyrin profile from COVID-19 patients. This accumulation cannot be attributed to pneumonia since the COVID-neg and pre-COVID cohorts show similar levels for all porphyrins except URO-I that accumulates slightly in the COVID-neg cohort. NMR analysis showed that neither aminolevulinate nor porphobilinogen accumulate.¹⁰ This signature is specific for COVID-19 patients and does not match the one found in porphyrias, specifically in those where the central enzymes of the haem pathway are malfunctioning due to deleterious mutations.^{11,12} Instead, in COVID-19, it is hard to rationalize how the pathological haem shortage may affect its own regulation, while the reported hyperferritinaemia may be a consequence of the inflammation process. Anyway, the non-productive byproducts URO I and COP I accumulate, inhibiting the central enzymes of the pathway and exacerbating haem shortage (unpublished results). This unstable scenario inevitably leads to the accumulation of porphyrins, as shown in Fig 1A (thick arrows).¹³ Incidentally, the normal levels of protoporphyrin IX (PROTO IX) suggests that SARS-CoV-2 is not directly competing with the haem group for the iron atom, as previously suggested.¹⁴ Porphyrin accumulation in COVID-19 sera is lower than in the blood of porphyria patients, in line with the limited duration of the viral infection episode.

Liver damage is often associated to mitochondrial dysfunction due to the deficient functioning of the electron transport chain (ETC) upon haem shortage.¹⁵ Ketone bodies (acetoacetic acid, 3-hydroxybutyric acid and acetone) were highly elevated in the serum of COVID-19 patients, which may be an adaptation to meet the reduced need for NADH, and consequently for acetyl-CoA, to feed into the ETC in

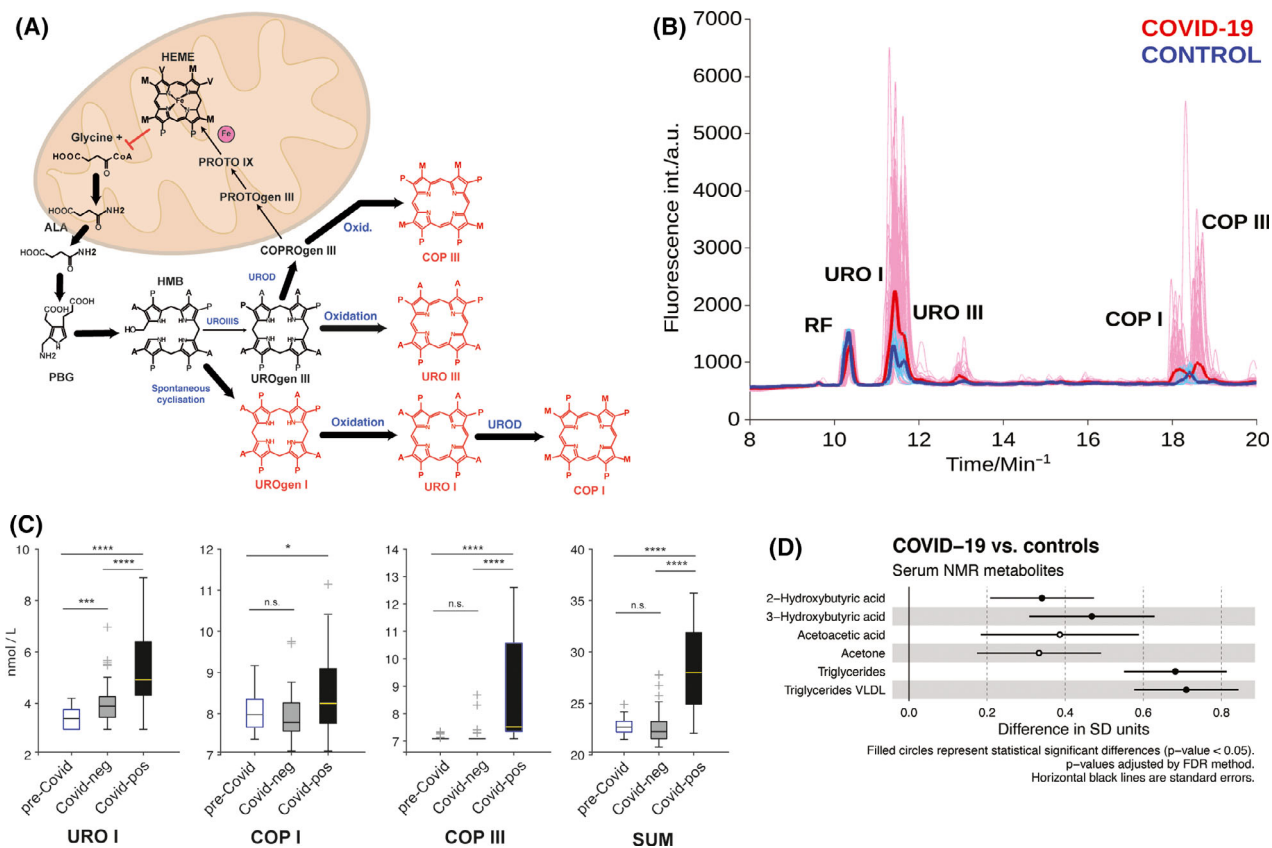


Fig 1. (A) The haem biosynthesis pathway. The enzymatic reactions for the haem production are shown in black; likewise, compounds in red are generated by-products. Thick arrows indicate reactions that are upregulated during erythropoiesis. A = acetyl; M = methyl; P = propionyl; V = vinyl. (B) High-performance liquid chromatography (HPLC) results of COVID-19 patients (red) and control individuals (blue). Solid lines correspond to the average intensity values in the chromatography whereas the coloured shadows indicate the standard deviation. Rivoflavin (RF) (10.3 min), uroporphyrin (URO) I (11.3 min), URO III (11.5 min), coproporphyrin (COP) I (18.0 min) and COP III (18.6 min) are easily detected. (C) Box-plot representation of the indicated porphyrin concentrations found in the pre-COVID, COVID-neg and the COVID-pos patient sera. *P* values: *, 0.01; ***, 0.0001; ****, <0.0001; n.s., not statistically significant. (D) Summary of statistical analysis for a selection of metabolites and lipoprotein subclasses of interest. Metabolite mean concentrations (pre-COVID): 2-hydroxybutyric, 23 mmol/l; 3-hydroxybutyric acid, 267 mmol/l; acetoacetic acid, 11 mmol/l; acetone, 28 mmol/l; triglycerides, 110 mg/dl; TG-VLDL, 32 mg/dl. Points represent the average increase (positive) or decrease (negative), in standard deviation (SD) units, for individuals with COVID-19, after controlling for gender and age. Filled points indicate that the value is significantly different from zero (*P* < 0.05). Horizontal black lines represent the 95% confidence interval.

these patients. As ketone bodies are produced from fatty acid oxidation (FAO)-derived acetyl-CoA, the increased content of triglycerides (TG) and very-low-density lipoproteins

(VLDL) observed may also be explained as an adaptation to meet the reduced need for FAO and prevent the intrahepatic accumulation of TG in these patients. We also found

Table I. Metadata from the individuals analysed.

	Pre COVID (n = 54)	COVID-neg (n = 60)	COVID-pos (n = 134)	<i>P</i> value	<i>n</i>
Main info					
Gender (female)	26 (48.15%)	30 (50.00%)	55 (41.04%)	0.435	248
Age (years)	61.02 ± 11.63	76.48 ± 8.48	67.07 ± 17.17	<0.001	248
Total hospitalization days	0 (0.0%)	n.a.	15.93 ± 22.33		134
Days in ICU	0 (0.0%)	n.a.	7.67 ± 20.13		134
Smoker	14 (25.93%)	n.a.	5 (3.73%)	<0.001	188
Pneumonia					
Unilateral	0 (0.0%)	9 (15.0%)	11 (8.40%)		131
Bilateral	0 (0.0%)	51 (85.0%)	104 (79.39%)		
Death	0 (0.0%)	n.a.	12 (8.96%)		134

elevated levels of 2-hydroxybutyric acid (Fig 1D), which is synthesized principally in the liver and released as a byproduct of glutathione synthesis by the transsulphuration pathway. An elevated concentration of the metabolite may reflect an increase of oxidative stress in COVID-19 patients. In brief, these results are compatible with impaired hepatic mitochondrial function in COVID-19, also consistent with the accumulation of porphyrins.


In summary, we here report an abnormal accumulation of porphyrins in association with severe COVID-19 that may shed some light on the haematological disorder associated with this devastating disease.

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Author contributions

EA, MS and AGV collected the samples; ISJ, CB, MB, GB, AL, PU, JG and TD performed the experiments; RG and NE performed the statistical data analysis; JMM and OM designed the research and OM wrote the paper.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Supplementary material.

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