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# Dysregulation of glutamine/glutamate metabolism in COVID-19 patients: a metabolism study in African population and mini meta-analysis

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**Abstract**

Coronavirus disease 2019 (COVID-19) remains a serious global threat. The metabolic analysis had been successfully applied in the efforts to uncover the pathological mechanisms and biomarkers of disease severity. Here we performed a quasi-targeted metabolomic analysis on 56 COVID-19 patients from Sierra Leone in western Africa, revealing the metabolomic profiles and the association with disease severity, which was confirmed by the targeted metabolomic analysis of 19 pairs of COVID-19 patients. A meta-analysis was performed on published metabolic data of COVID-19 to verify our findings. Of the 596 identified metabolites, 58 showed significant differences between severe and non-severe groups. The pathway enrichment of these differential metabolites revealed glutamine and glutamate metabolism as the most significant metabolic pathway (Impact=0.5; -Log10P=1.959). Further targeted metabolic analysis revealed six metabolites with significant inter-group differences, with glutamine/glutamate ratio significantly associated with severe disease, negatively correlated with 10 clinical parameters and positively correlated with $SPO_2$ ($r_s$=0.442, P=0.005). Mini meta-analysis indicated elevated glutamate was related to increased risk of

COVID-19 infection (pooled OR=2.02; 95% CI: 1.17–3.50) and severe COVID-19 (pooled OR=2.28; 95% CI: 1.14–4.56). In contrast, elevated glutamine related to decreased risk of infection and severe COVID-19, the pooled OR were 0.30 (95% CI: 0.20–0.44), and 0.44 (95% CI: 0.19–0.98), respectively. Glutamine and glutamate metabolism are associated with COVID-19 severity in multiple populations, which might confer potential therapeutic target of COVID-19, especially for severe patients.

Key words: glutamine; glutamate; metabolism; COVID-19, biomarkers, meta-analysis

## Introduction

Despite advances in public health interventions, antiviral drugs development and multiple vaccination strategies, coronavirus disease 2019 (COVID-19) remains a serious global threat. By the end of March 2022, over 469,212,705 confirmed cases with 6,077,252 deaths (overall mortality rate 1.29%) worldwide were reported to World Health Organization (WHO) (https://covid19.who.int/). COVID-19 ranged widely from asymptomatic and unspecific symptoms to severe respiratory distress and multiorgan failure or even death[1, 2]. The severity rate of COVID-19 ranged from 5% to 20% worldwide[3]. Based on the largest cohort study that recruited 44,415 COVID-19 patients in China, 14% (6,188 cases) were severe and 5% (2,087) were critical[4]. The mortality rate in patients who required

mechanical ventilation (severe) reaches up to 88.1%, which is much higher compared with patients who did not receive mechanical ventilation (non-severe, mortality rate 11.7%)[3].

The early diagnosis and prognosis of COVID-19 are important for proper healthcare resource allocation and treatment of severe patients, this was greatly hindered by the lack of an appropriate prognostic indicators for early recognition of adverse outcome. Metabolomic phenotyping, the simultaneous measurement of multiple small molecules in a biological sample, can provide a comprehensive assessment of an individual's biochemical status. Application and integration of metabolomic technologies was one of the most intensively explored approach to demonstrate the metabolic status of COVID-19 patients, to unravel underlying pathogenic mechanism and to identify valuable biomarkers and therapeutic approaches for COVID-19[5].

Based on infected cells, tissues, and body fluids from patients and by comparison with healthy individuals, multiple metabolic signatures and potential metabolite-biomarkers of COVID-19 patients were discovered [5, 6]. To great extent, the observed metabolite changes indicative of significant metabolic dysregulation are correlated with dysregulation of the immune function, coagulation and complement system[7-9]. The most notable and distinctive metabolic reprograming was seen for amino acids, for example, arginine and tryptophan were altered as a result of SARS-COV2 infection in different studies, all closely linked to immuno-dysfunction and vasculopathy process of COVID-19 [10-12].

Such studies had been scarcely performed in Africa.

Africa as the world's most under-developed regions, to identify COVID-19 might be challenging owing to its limited resources to establish surveillance and diagnosis systems, particularly in countries with low socioeconomic background. Until recently, only rare evidence about the clinical impact of COVID-19 comes from Africa, and most of them are reported from South Africa, where more than 3,818,125 cases, more than 100,471 deaths, with a case fatality rate of 2.63% have been reported by May 9, 2022 (https://covid19.who.int/). Since 2014, our team had participated in the urgent medical aid for Ebola disease in Sierra Leone, and with the onset of the COVID-19 pandemic, we were able to test and treat SARS-CoV-2 infection in the 34$^{th}$ Military Hospital of Republic of Sierra Leone Armed Forces hospital, which was designated as one of the medical facilities to treat COVID-19.

Here, to determine the metabolomic change of COVID-19 patients from population in Africa, we performed quasi-targeted and targeted metabolomic analysis on severe and matched non-severe patients. The aberrant glutamine and glutamate metabolism was determined as one of the most significant metabolic phenotypes in severe COVID-19 patients. In order to confirm the diagnosis and prognosis value of the glutamine and glutamate for COVID-19 in wider population, we performed a meta-analysis to assess the association between glutamate, glutamine, and the risk of severe COVID-19.

## Methods

### Study design, participants, and data collection

This observational study was performed in Infectious Disease Prevention and Control Center in the 34th Military Hospital of Republic of Sierra Leone Armed Forces. The COVID-19 patients diagnosed according to positive SARS-COV-2 RNA based on the WHO interim guidance (https://www.who.int/publications-detail/) were recruited from April to November, 2020. The clinical information was collected and routine laboratory tests was conducted from these patients during whole disease course. Severe COVID-19 patients referred to cases that fulfilled any of the following criteria: 1) dyspnea, respiratory rate ≥ 30/min; 2) blood oxygen saturation ≤ 93% and ratio of partial pressure of arterial oxygen to fraction of inspired oxygen < 300; and 3) lung infiltrates > 50% within 24 to 48 h. A confirmed case with mild symptoms without presenting any of the criteria was defined as non-severe case. Information was collected from individual patient including demographic characteristics (age, gender, ethic, medical conditions), clinical information, and all laboratory test results during hospitalization. All information in electric medical records were entered into an EpiData database (EpiData Association, Odense, Denmark) by trained staff. All procedures were performed in accordance with the ethical standards of the institutional review board of the Sierra Leone Ethics and Scientific Review Committee and the Declaration of Helsinki. Informed consent was obtained from all participants and/or their guardians.

**Liquid chromatography-tandem mass spectrometry (LC-MS) measurement.** Within 24 h after hospital admission, the whole blood samples were collected into pro-coagulation tube, centrifuged (2000 × g for 15 min) within 30 mins, with the serum separated and prepared for LC-MS assay. Briefly, the serum was treated by acetonitrile at room temperature and then centrifuged at 14000 rpm for 10 min at

4°C. The supernatants were pipetted out and stored at 4°C before detection. From each patient, 10 μL of serum samples were pooled and used as the quality control (QC) samples, which was pretreated in parallel with the testing samples.

**LC-MS analysis**

The metabolomic analysis of testing and QC samples was performed as previously described [13]. Briefly, high-performance liquid chromatography separation was performed by using an Ultimate 3000 LC system (Thermo Scientific, Waltham, MA, USA) coupled with an Acquity UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 um; Waters Corporation, Milford, MA, USA). The mass spectrometry was performed in both the positive and negative electrospray ionization modes (ESI + and ESI -) using an Orbitrap Elite mass spectrometer (Thermo Scientific Waltham, MA, USA). The QC samples were tested at regular intervals (every 10 samples) during the whole analysis process.

Targeted metabolite profiling of glutamine related metabolites including organic acids and polyamines in serum samples was performed as previously described [14, 15]. Solvent mixtures containing acetonitrile and water were used to extract organic acids. For polyamine extraction, solution containing methanol, water and formic acid was used to treat serum samples. These metabolite extracts were analyzed using LC-MS according to the procedure described above with internal standards of each concerned metabolites.

**Metabolomic data analysis and pathway enrichment analysis**

All metabolic analysis were conducted using MetaboAnalyst 5.0 online tool (https://www.metaboanalyst.ca/). First, the data were log transformed and auto-scaled (Supplement Figure 1), then multidimensional statistical analyses was performed by applying orthogonal partial least square discriminant analysis

(OPLS-DA). The influence intensity of metabolites was measured by the variable importance in the projection (VIP) obtained from the OPLS-DA model, and metabolites with a VIP value > 1.5 were selected as potential differential metabolites. Hierarchical clustering of all potential metabolites was performed after unit variance scaling for each metabolite. Pathway enrichment analysis were based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and the human metabolome database (HMDB).

**Statistics**

Continuous variables were described with means and standard deviations (SD) or with medians and interquartile range (IQR). Categorical variables were described with frequencies and proportions. Student's t-tests were adopted to compare the mean levels between two groups if the variable was normally distributed, otherwise, the Mann-Whitney U test were adopted. Chi-square tests or Fisher exact test were adopted to compare the percentages of categorical variables between groups. Wilcoxon signed-rank test was used to compare the relative content of metabolites between paired samples. Spearman rank correlation analysis was applied to assess the correlation between metabolites and clinical parameters. Statistical analysis was performed using R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). A two-sided $p < 0.05$ was considered statistically significant.

**Mini meta-analysis of glutamate and glutamine in relate to COVID-19**

A literature search was performed from the PubMed, and Web of Science up to Dec 2021, by using the following search terms: (multiomics or metabolomics or metabolism) and (COVID-19 or SARS-Cov-2). The references of the included studies were further reviewed to identify additional studies. Studies were included

in this meta-analysis if they met the following criteria: (1) case control study design with study subjects grouped as COVID-19 (+) / COVID-19 (-) or severe COVID-19/ non-severe COVID-19; (2) the metabolomic data were acquired from blood samples; (3) the raw data were provided; (4) metabolomic data include the level glutamate or glutamine. Two investigators (X.L. and X.Z.) reviewed the studies independently, and for any inconsistency, a consensus was achieved by consulting a senior reviewer (W.L.).

To address the concern of incomparable spectral peaks across different studies due to various chromatographic and/or MS conditions, ROC analysis was performed for each study to calculate the optimal critical values of glutamate and glutamine for COVID-19 (+) / COVID-19 (-) or severe / non-severe. For each study, the patient was assigned into high or low group according to the cut-off value of the glutamate and glutamine, and the association with disease (COVID-19 v.s non-OVID-19) and disease outcome (severe v.s non-severe) were calculated by using OR and 95% CI. From each study, the following data were extracted by the three investigators (X.L., W.X. and J.C.): (1) the first author; (2) study year; (3) study site; (4) sample type; (5) test method; (6) sample size; (7) metabolite results; (8) OR with 95% CI. We weighted the study-specific log ORs by inverse variance, to calculate pooled ORs with corresponding 95% CI of the association between glutamate and glutamine and the risk of disease or severe disease of COVID-19. The $I^2$ was used to assess heterogeneity ($I^2$ values of 0, 25, 50, and 75% represented no, low, moderate, and high heterogeneity respectively)[16]. The fixed-effect model was used as the pooling method if moderate or lower heterogeneity ($I^2 \leq 50\%$) was found; otherwise ($I^2 > 50\%$), the random-effect model was adopted. All statistical analyses were performed with STATA version

15.0 (Stata Corporation, College Station, TX, USA). A 2-tailed $p \leq 0.05$ was considered statistically significant.

## Results

### Study design and patient

From April 2, 2020 to July 26, 2020, altogether143 patients diagnosed with COVID-19 was enrolled in the study, 30 cases with admission delay over 15 days and 22 cases without adequate clinical information were excluded (Figure 1A). The quasi-targeted metabolomics method was firstly used to analyze the serum samples collected from 56 COVID-19 confirmed cases (28 patients with severe COVID-19 *VS.* 28 age and gender matched patients with non-severe COVID-19). Of the 56 patients, 89.30% were male, the mean age was 53.14 (SD, 13.45), with a mean interval of 7.30 (SD 3.67) days from disease onset to test performed, which were balanced between the severe and non-severe patients (Table 1). Significant differences in white blood cell (WBC), monocyte and neutrophil count, C-reactive protein (CRP), albumin, total and direct bilirubin (TBIL and DBIL), etc. were identified between severe and non-severe COVID-19 patients.

### Dysregulated glutamine/glutamate metabolism in severe COVID-19 patients

Altogether 596 metabolites were able to be identified (out of 2200+ metabolites) by alignment to the self-built database in the quasi-targeted metabolomics analysis. By OPLS-DA, the metabolomics profiles could clearly distinguish severe from non-severe COVID-19 patients at acute infection stage (Figure 1B). 58 inter-group differential metabolites (VIP >1.5) were determined (Supplement

Table 1), based on which distinct metabolic features among groups were demonstrated by hierarchical clustering (Figure 1C). Further pathway enrichment analysis suggested glutamine and glutamate metabolism to be the prominent dysregulated metabolic pathways in severe COVID-19 patients (Figure 1D). Specifically, a significant lower level of glutamine and higher level of glutamate, which corresponded with a significant reduction in the glutamine/glutamate ratio, were observed among severe COVID-19 patients (Figure 1E), implicating the downregulated glutamine bioavailability and enhanced glutaminolysis effect as relate to the severe COVID-19.

Glutamate could be converted into α-ketoglutarate (α-KG), and α-KG participated in tricarboxylic acid (TCA) cycle. Although glutamate was significantly elevated in severe patients, α-KG were comparable between severe and non-severe COVID-19 patients. Succinate as an indirect product of glutamate, was otherwise decreased in severe patients compared to that of non-severe patients (Figure 2). The ammonia transfer between glutamine and glutamate was also involved in conversion between aspartate and asparagine. In congruence with altered glutamate and glutamine, we found elevated asparagine and loss of aspartate in severe COVID-19 patients as well (Figure 2).

**The glutamine/glutamate metabolism in relate to clinical parameters**

To confirm the changes of glutamine/glutamate and related metabolites, we performed an absolute quantification by targeted metabolomics analysis. Altogether 28 glutamine/glutamate associated metabolites were evaluated on 19

pairs of matched severe and non-severe COVID-19 patients, the detailed results was shown in Supplement Table 2. (Demography and clinical data presented in Supplement Table 3).

The glutamine/glutamate ratio was related to clinical parameters of routine test that were evaluated from the sample collected at the same point as that for the metabolomic analysis. A significant negative association was observed between glutamine/glutamate ratio and the level of DBIL($r_s$=-0.623, P=0.001), CRP($r_s$=-0.603, P=0.001), WBC count ($r_s$=-0.489, P=0.003), neutrophil count ($r_s$=-0.473, P=0.005), LDH ($r_s$=-0.576, P=0.008), ALT ($r_s$=-0.432, P=0.009), TB ($r_s$=-0.382, P=0.022), BUN ($r_s$=-0.344, P=0.040), D-dimer ($r_s$=-0.386, P=0.042), and a positive correlation with $SPO_2$($r_s$=0.442, P=0.005) (Figure 3A), reflecting the glutaminolysis effect may be correlated with immune responses, organ dysfunction, and coagulation dysregulation of COVID-19 and being an indicator of systematic function of COVID-19 patients. In addition to glutamine/glutamate, there were six metabolites that were differentially expressed between severe and non-severe COVID-19 patients, with the most prominent difference observed for L−Citrulline, Serotonin, and Polyamines (Figure 3B).

**Mini meta-analysis on glutamine/glutamate metabolism of COVID-19**

The search strategy identified 357 literatures from PubMed, and 153 from Web of Science. After excluding 306 irrelevant articles by reading titles and/or abstract and 37 literatures by reading the full text, 18 articles that involved 3,177 participants were included in the meta-analysis. The detailed characteristics of the

included studies are shown in Supplement Table 4. Seven of them were conducted in North America (NA), four in Asia, six in Europe, and one in Oceania. Three types of samples were involved, with eight using plasma sample, nine using serum and one using red blood cells (RBCs). Eight studies were performed based on Ultra-High-Pressure Liquid Chromatography–Mass Spectrometry metabolomics (UHPLC-MS), five on LC-MS, five on other methods, such as Nuclear Magnetic Resonance (NMR), Gas Chromatography–Mass Spectrometry (GC-MS).

Studies that provided original data were used to evaluate the relationship between the COVID-19 infection and the level of glutamate or glutamine (in 16 studies), between the disease severity and the level of glutamate (in six studies) or glutamine (in eight studies). The pooled OR of glutamate for the risk of COVID-19 infection was determined to be 2.02 (95% CI: 1.17 – 3.50; $I^2$ = 86.1%) (Figure 4A), and for the risk of severe COVID-19 was 2.28 (95% CI: 1.14 – 4.56; $I^2$ = 65.5%) (Figure 4C). These results indicated that elevated glutamate was related to an increased risk of COVID-19 infection and severe COVID-19. In contrast, the elevated level of glutamine was related to a decreased risk of infection and severe COVID-19, with the pooled OR of glutamine for the risk of COVID-19 infection and severe COVID-19 estimated as 0.30 (95% CI: 0.20 – 0.44; $I^2$ = 68.0%) (Figure 4B), and 0.44 (95% CI: 0.19 – 0.98; $I^2$ = 80.2%) (Figure 4D), respectively.

**Discussion**

Several previous studies based on the metabolome profiling have emphasized the impairment of amino acid metabolism in COVID-19[6]. Our metabolome results

further showed significantly decreased glutamine and elevated glutamate of severe COVID-19 patients compared to non-severe patients. The core metabolites of this metabolism, glutamine and glutamate were correlated with hematological, inflammatory and organ dysfunction of the patients, corroborating the important role of glutamine and glutamate metabolism during the clinical process. Via meta-analysis of previously published metabolomic results of COVID-19 patients, we further verified the finding that glutamine and glutamate metabolism was significantly altered in COVID-19 patients, especially in severe patients.

Glutamine is a non-essential amino acid involved in multiple physiological metabolic function, such as energy generation via TCA cycle, lipid and purine synthesis, and glutathione production[17]. Glutaminase converts glutamine to glutamate, with the latter generating α-ketoglutarate to enter the TCA cycle for ATP production. Glutamine/glutamate metabolism was also involved in production of other macromolecules. The ammonia transfer from glutamine to glutamate is also involved in the balance of asparagine and aspartate[18]. And aspartate was the precursor of purine and pyrimidine[19] that could be used to synthesize viral RNA and DNA. Furthermore, glutamine/glutamate enables the formation of citrulline and other intermediate compounds in urea cycle, and glutamine/glutamate are also precursors of glutathione, an antioxidant compound that plays an important role in preventing oxidative stress in severe infection[20]. Taken together, glutamine metabolism provides metabolic intermediates that are required for viral proliferation, including energy supply, anaplerosis, virus

assembly, cell growth, and cell survival [21], therefore is highly likely manipulated to support the viral replication. Actually, the altered glutamine utilization and metabolism had been previously reported in multiple virus infection. In cells infected by HSV-1, VACV, the glutamine-dependent anaplerotic pathway is activated to sustain energy production and nucleotide synthesis for viral replication[22, 23]. Deprivation of glutamine leads to impairments of VACV replication[24]. Inhibition of glutamine metabolism reduced or inhibited viral replication such as coxsackie B3 virus[25], HCV[26].

COVID-19 patients, especially severe COVID-19 patients, showed both reduced level of glutamine and elevated level of glutamate which indicated increased utilization of glutamine[19]. However, the detailed association between glutamine and glutamate level with disease severity remained undetermined, and other related metabolites was not examined simultaneously. Our finding was also in line with previous studies on metabolic analysis [27, 28], where metabolites in glutamine/glutamate related metabolic pathways were likewise significantly altered, featured by elevated asparagine, reduced aspartate, succinate and citrulline among severe COVID-19 patients compared with non-severe patients.

Before the current study, numerous indicators that highlighted hematological abnormalities (e.g. reduced Lymphocytes and their subsets), tissue damage (e.g. abnormally altered LDH, AST, ALT, and other biochemical parameters), coagulation dysregulation (e.g. increased D-dimers and fibrinogen levels), clinical inflammatory overexpression (increased CRP, PCT and ferritin) and

immunological and transcriptomic signatures (proinflammatory cytokines such as IL-1 alpha, IL-6, and IL-8 et al) had been implicated for their usage as biomarkers of disease severity of COVID-19 [29, 30]. Based on high through-put RNA sequencing technology, many genetic signatures were identified to be associated with COVID-19 disease progression, with most of them involved in IFN responses, cell death, inflammation, and regulation of immune cell function. Glutamine metabolism on the other hand, was supposed to participates in vast majority of above-mentioned pathways, process of immune regulation or cellular functions. For example, the glutamine metabolism is required for B cell proliferation, germinal center formation and memory B cell generation[31]. Glutamine regulates T cell proliferation and affects the differentiation of naïve CD4+ T cells to Th1 cells[32], and it's also crucial for the fate decision of Th17/Treg[33]. α-KG, a production of glutaminolysis, is reported to promote alternative activation of macrophage and ameliorates inflammation[34]. Glutamine promotes neutrophil phagocytosis and bacterial killing activities[35, 36]. The clinical application of glutamine in HIV infected patients, increased the number of CD4+ T cells and decreased the serum levels of C-reactive protein, interferon-inducible gamma protein-10, regulated on activation normal T cell expressed and secreted (RANTES), and macrophage inflammatory protein-1b[19]. Furthermore, a clinical trial showed that oral glutamine supplementation shortened the length of hospital stay and reduced the need for intensive care in patients with COVID-19[37]. In addition, some investigators proposed that inhibitors of

glutamine metabolism, like Azaserine and 6-diazo-5-oxo-L-norleucine, instead of glutamine supplementation seems to be a better strategy to reduce or abolish viral replication[19]. But whether glutamine supplementation or inhibitors of glutamine metabolism could restore immune function of COVID-19 patients or show a direct anti-SARS-COV2 effect requires further investigation.

Long COVID-19 is emphasized by researchers. Recent surveys showed that it influenced approximately 14% adults[38] and approximately 21% children and adolescents[39]. The frequently reported symptoms of long COVID-19 include headache, anosmia, dyspnea, and diarrhea et al[40]. The underlying biological mechanism remains to be explored. Hyper-inflammation, oxidative stress, and endothelial cell injury have been suggested as the drivers of long COVID-19[41, 42]. As a pluripotent biological process, glutamine/glutamate metabolism is involved in immune regulation[43] and oxidative stress[44]. Supplementation of glutamine analogue also attenuates endothelial cell injury[45]. In COVID-19 patients, administration of glutamine decreased levels of inflammatory cytokines and oxidative stress indicator, and increased total antioxidant capacity[46]. The role of glutamine/glutamate metabolism in pathogenesis of long COVID-19 remains to be investigated and it is a promising therapeutic target to alleviate symptoms of long COVID-19.

Our data shed light on the molecular changes reflected in COVID-19 sera, which could potentially yield critical diagnostic markers or therapeutic targets for managing severe COVID-19 patients. The application of the glutamine metabolites as a powerful prognosis in adverse outcome and the antiviral effect of

glutamine supplementation could be well supported. Several drug classes, which bear the activities to deplete glutamine (L-asparaginase), inhibit glutaminase (968, BCB839, and PTES), suppress glutamate dehydrogenase (EGCG and R162), inhibit aminotransferase (AOA), and mimic glutamine (DON, azaserine, and acivicin), have shown antiviral activities[19]. Whether this could pose as a therapeutic approach in COVID-19 needed further investigation.

The present study was subject to major limitations that an observational study design was applied, therefore no causality can be inferred between metabolites perturbation and COVID-19 disease progression. The metabolite profiles of COVID-19 were obtained in the serum samples to reflect the systematic change that ensued from SARS-COV-2 infection, while metabolite profiling on the respiratory samples might be needed to offer an in-depth understanding of the pathogenesis. Since the respiratory tract is the core site of initial SARS-CoV-2 infection and the most common site of serious clinical manifestations[47, 48] and several studies have showed that there were significant differences in the responses of peripheral blood and respiratory samples post SARS-CoV-2 infection[49-51].

Despite of these limitations, the current study for the first time identified glutamine and glutamate metabolism to be associated with COVID-19 severity in African populations, with the findings corroborated by accumulating data from studies performed in other ethnics than the African. Our data shed light on the ubiquitous change of glutamine and glutamate metabolite in COVID-19 sera, which could potentially yield critical diagnostic markers or therapeutic targets for

managing severe COVID-19 patients.

**Conflict of Interest Disclosure**

All authors declare no competing interest.

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**Data Availability Statement**

Data available on request from the authors

**Contributions**

X.L, B.T, X.Z, W.X, P.H, R.C, M.J, W.L, and W.C participated in the study design and data collection. B.X, J.Z, J.C, Q.L, C.L and S.S analyzed the data and wrote the manuscript. X.L, S.S, W.L and W.C critically reviewed the manuscript. All participated authors critically reviewed the manuscript.

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**Figure legends**

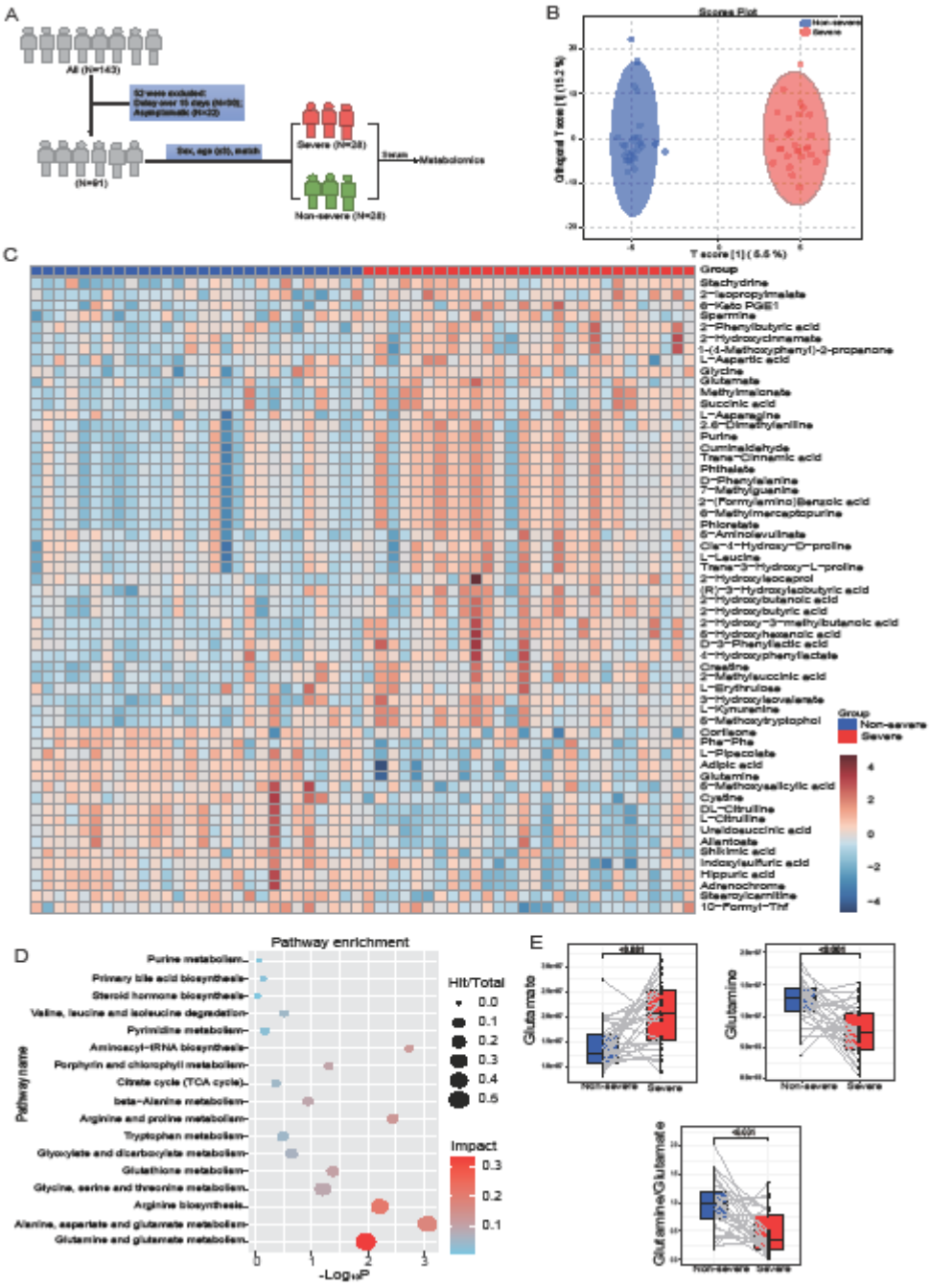



Figure 1. Quasi-targeted metabolomics analysis of patients with COVID-19. (A) Fifty-six patients (28 with severe COVID-19 and 28 with non-severe COVID-19). (B) The serum metabolic phenotypes of severe COVID-19 patients substantially differed from non-severe patients by OPLS-DA. (C) Heatmap of 58 potential metabolism which might have an impact on COVID-19 severity. (D) Metabolic set enrichment analysis using the significantly enriched metabolites between

severe and non-severe COVID-19 patients. (E) Glutamine, glutamate, and the ratio of glutamine and glutamate were significantly different between severe and non-severe COVID-19 patients.

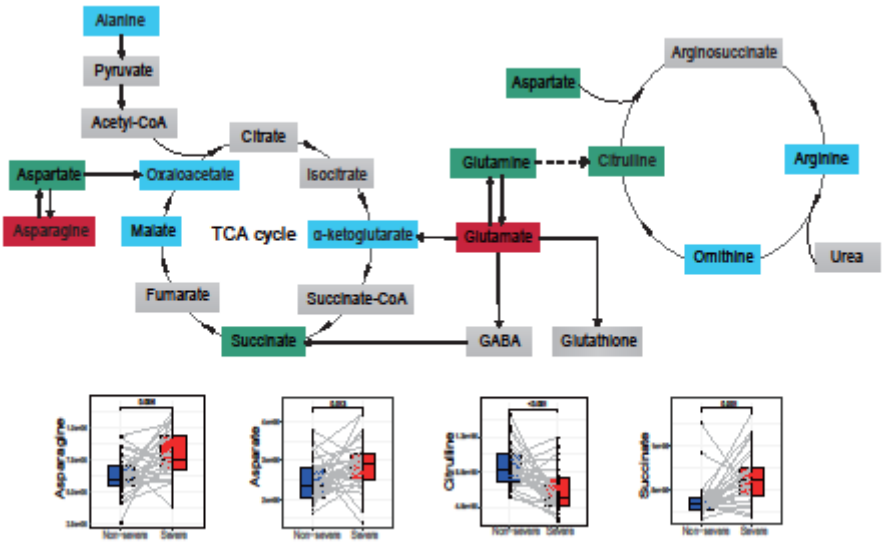
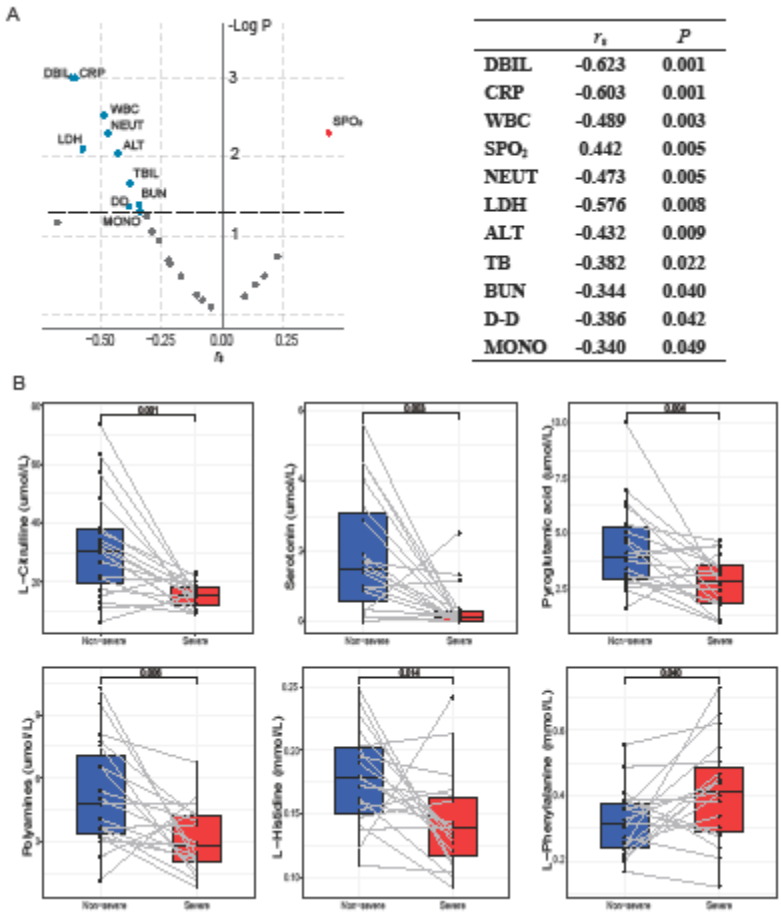



Figure 2. Disturbed metabolisms associated with glutamine/glutamate metabolism in severe COVID-19 patients. Red represents the up-regulated metabolites in severe COVID-19, green represents the down-regulated metabolites in severe COVID-19, blue represents the unchanged metabolites, and gray represents the undetected metabolites.




| | $r_s$ | $P$ |
|---|---|---|
| DBIL | -0.623 | 0.001 |
| CRP | -0.603 | 0.001 |
| WBC | -0.489 | 0.003 |
| $SPO_2$ | 0.442 | 0.005 |
| NEUT | -0.473 | 0.005 |
| LDH | -0.576 | 0.008 |
| ALT | -0.432 | 0.009 |
| TB | -0.382 | 0.022 |
| BUN | -0.344 | 0.040 |
| D-D | -0.386 | 0.042 |
| MONO | -0.340 | 0.049 |

Figure 3. Targeted metabolomics analysis of patients with COVID-19. (A) Correlation between glutamine/glutamate ratio and laboratory parameters and vital signs. Correlation coefficient was calculated by spearman rank correlation analysis. (B) Additional differential metabolites between severe and non-severe COVID-19 patients.

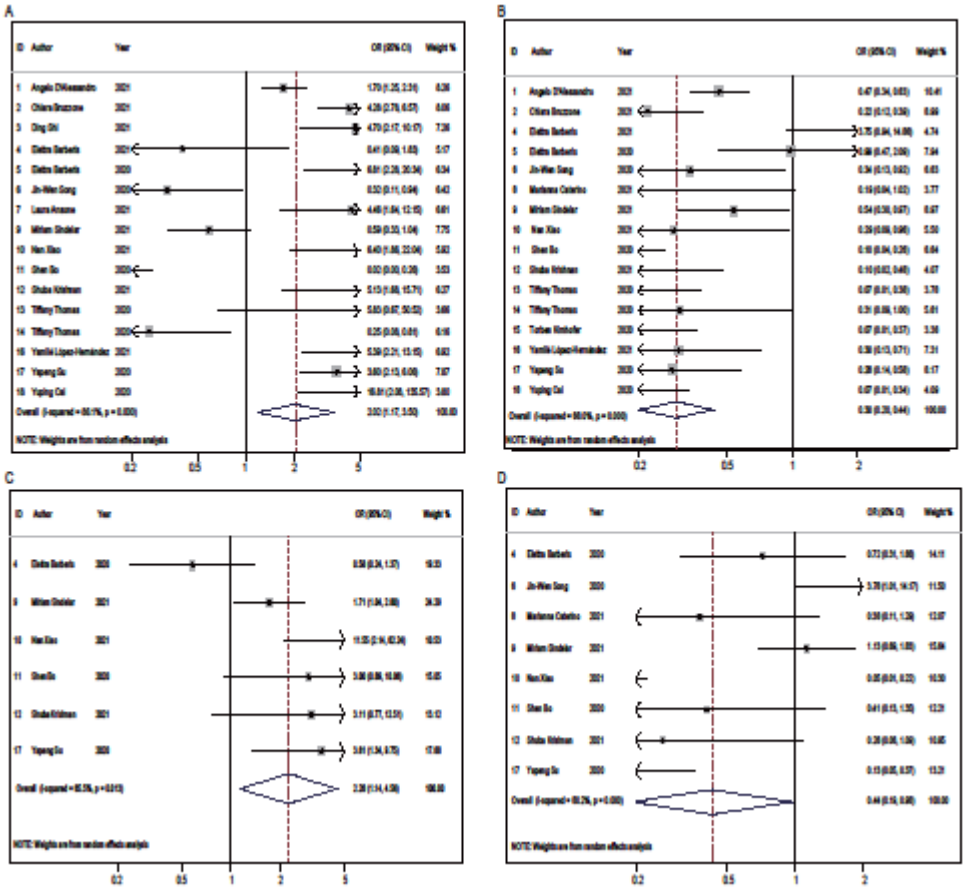


Figure 4. Forest plot of the odds ratio (ORs) with 95% confidence intervals (CIs) of the association between glutamate (A), glutamine (B) and the risk of infection; the association between glutamate (C), glutamine (D) and severity of COVID-19.

SFigure 1. Distribution of metabolite before and after data transformation.

Table 1. Baseline characteristics of COVID-19 patients

| | Overall (N=56) | Non-severe (N=28) | Severe (N=28) | *P* |
|---|---|---|---|---|
| Sex, n (%) | | | | 1.000 |
| Male | 50 (89.30) | 25 (89.30) | 25 (89.30) | |
| Female | 6 (10.70) | 3 (10.70) | 3 (10.70) | |
| Age, mean (SD), years | 53.14 (13.45) | 53.11 (13.57) | 53.18 (13.58) | 0.984 |
| Interval from onset to testing, mean (SD), days | 7.30 (3.67) | 7.25 (3.41) | 7.36 (3.98) | 0.914 |
| Laboratory parameters | | | | |
| WBC, median [IQR], $10^9$/L | 8.50 [5.27, 12.17] | 5.40 [4.55, 7.75] | 11.90 [9.50, 14.60] | **<0.001** |

| | | | | |
|---|---|---|---|---|
| Lymphocyte, median [IQR], $10^9$/L | 1.70 [1.28, 2.40] | 2.20 [1.40, 2.50] | 1.60 [1.20, 1.90] | 0.115 |
| Monocyte, median [IQR], $10^9$/L | 0.40 [0.30, 0.70] | 0.30 [0.30, 0.50] | 0.60 [0.40, 0.90] | **0.001** |
| Neutrophils, median [IQR], $10^9$/L | 5.75 [2.98, 9.62] | 3.40 [2.40, 4.90] | 9.60 [7.40, 11.80] | **<0.001** |
| HGB, median [IQR], g/L | 145.50 [127.75, 157.00] | 145.00 [129.00, 151.50] | 146.00 [122.00, 166.00] | 0.458 |
| PLT, median [IQR], $10^9$/L | 162.50 [126.00, 225.25] | 152.00 [110.00, 212.50] | 191.00 [130.00, 226.00] | 0.170 |
| ALB, median [IQR], g/L | 40.10 [36.25, 46.50] | 45.20 [38.08, 49.00] | 37.90 [34.75, 41.15] | **0.002** |
| TP, median [IQR], g/L | 73.20 [67.45, 76.90] | 72.55 [67.53, 75.82] | 74.40 [67.95, 78.15] | 0.596 |
| ALT, median [IQR], U/L | 38.00 [23.55, 59.80] | 32.85 [22.18, 49.95] | 46.20 [26.90, 73.00] | 0.170 |
| AST, median [IQR], U/L | 29.60 [25.90, 37.10] | 29.80 [26.30, 33.80] | 29.30 [25.77, 45.75] | 0.310 |
| TBIL, median [IQR], umol/L | 13.70 [11.85, 20.30] | 12.55 [10.80, 16.30] | 14.30 [12.90, 21.85] | **0.040** |
| DBIL, median [IQR], umol/L | 6.50 [5.20, 8.35] | 5.65 [4.77, 6.55] | 6.90 [6.25, 8.95] | **0.009** |
| GLU, median [IQR], mmol/L | 7.25 [6.10, 8.70] | 6.10 [5.80, 6.90] | 8.10 [7.50, 11.25] | **<0.001** |
| BUN, median [IQR], mmol/L | 6.30 [4.30, 11.00] | 4.40 [3.20, 6.30] | 7.85 [4.80, 11.17] | **0.006** |
| CREA, median [IQR], umol/L | 102.00 [86.00, 148.50] | 99.00 [86.00, 121.00] | 103.00 [90.50, 151.50] | 0.583 |
| UA, median [IQR], umol/L | 393.95 [295.28, 462.60] | 364.45 [295.28, 409.40] | 424.05 [308.25, 502.93] | 0.275 |
| LDH, median [IQR], U/L | 279.95 [196.43, 400.02] | 209.90 [178.55, 312.50] | 353.80 [261.88, 505.15] | **0.025** |
| CRP, median [IQR], mg/L | 70.76 [20.45, 114.47] | 20.45 [4.44, 65.68] | 103.72 [73.97, 134.10] | **<0.001** |
| D-D, median [IQR], mg/L | 2.74 [0.33, 8.78] | 0.33 [0.28, 1.78] | 8.12 [4.38, 15.00] | **<0.001** |
| Comorbidities, n (%) | 40 (71.40) | 16 (57.10) | 24 (85.70) | **0.038** |
| Symptoms and vital signs | | | | |
| Fever, n (%) | 37 (66.10) | 18 (64.30) | 19 (67.90) | 1.000 |
| Cough, n (%) | 34 (60.70) | 15 (53.60) | 19 (67.90) | 0.412 |
| Dyspnea, n (%) | 24 (42.90) | 3 (10.70) | 21 (75.00) | **<0.001** |
| Fatigue, n (%) | 26 (46.40) | 12 (42.90) | 14 (50.00) | 0.789 |
| Temperature, mean (SD), ℃ | 36.58 (0.49) | 36.52 (0.35) | 36.63 (0.60) | 0.435 |
| $SPO_2$, mean (SD), % | 91.64 (6.90) | 97.00 (1.61) | 86.29 (5.89) | **<0.001** |
| SBP, mean (SD), mmHg | 138.61 (26.08) | 130.75 (18.46) | 146.46 (30.28) | **0.023** |
| DBP, mean (SD), mmHg | 83.50 (15.24) | 80.79 (12.87) | 86.21 (17.10) | 0.185 |
| HR, mean (SD), /min | 95.18 (18.02) | 85.04 (13.99) | 105.32 (15.89) | **<0.001** |
| RR, mean (SD), /min | 24.25 (4.76) | 20.46 (2.66) | 28.04 (3.07) | **<0.001** |

SD: Standard deviation; WBC: white blood cell; IQR: interquartile range; HGB: hemoglobin; PLT: platelet; ALB: albumin; TP: total protein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; DBIL: direct bilirubin; GLU: glucose; BUN: urea nitrogen; CREA: creatine; UA: uric acid; LDH: lactate dehydrogenase; CRP: C-reactive protein; D-D: D-dimer; $SPO_2$: oxygen saturation; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; RR: respiratory rate.