Comparative metabolomic analysis reveals shared and unique features of COVID-19 cytokine storm and surgical sepsis

Russkikh Ia.V.[1], Popov O.S.[1,2], Klochkova T.G.[1,3], Sushentseva N.N.[1], Apalko S.V.[1,2], Asinovskaya A.Yu.[1,2], Mosenko S.V.[1,2], Sarana A.M.[2], Shcherbak S.G.[1,2]

[1] Saint Petersburg State Health Care Establishment the City Hospital No. 40 of Health Department of the Saint Petersburg Kurortniy District Administration, 197706, st. Borisova, 9, Sestroretsk, Russian Federation

[2] Saint-Petersburg State University, Government of the Russian Federation, 199034, Saint-Petersburg, Russian Federation

corresponding author: Klochkova T.G., Saint Petersburg State Health Care Establishment the City Hospital No. 40 of Health Department of the Saint Petersburg Kurortniy District Administration, 197706, st. Borisova, 9, Sestroretsk, Russian Federation; e-mail: TKlochkova@list.ru.

Abstract

Background and aims: The clinical manifestations of the cytokine storm (CS) associated with COVID-19 resemble the acute phase of sepsis. Metabolomics may contribute to understanding the specific pathobiology of these two syndromes. The aim of this study was to compare serum metabolomic profiles in CS associated with COVID-19 vs. septic after surgery patients.

Materials and methods: In retrospective cross-sectional study serum samples from patients with CS associated with COVID-19, with comorbidity (n=43) and without comorbidity (n=40) as well as serum samples from patients with surgical sepsis (n=41) were investigated. Serum samples from healthy volunteers (n=110) were used as a control. A targeted metabolomic analysis was carried out in all samples using LC-MS/MS method. The R programming language was used for statistical data processing.

Results: Analysis revealed that similar alterations in serum metabolome of patients with COVID-19 and in septic patients were associated with amino acid metabolism, nitrogen metabolism, inflammatory status, methionine cycle and glycolysis. The most significant difference was identified for the serum levels of metabolites of kynurenine synthesis, tricarboxylic acid cycle, as well as for gamma-aminobutyric acid and niacinamide. The metabolic pathway of cysteine and methionine metabolism was disturbed significantly in COVID-19 and septic patients.

Conslusion: For the first time, the similarities and differences between the serum metabolomic profiles of patients with CS associated with COVID-19 and patients with surgical sepsis were investigated. Identified common and different metabolic patterns were associated with amino acid metabolism, nitrogen metabolism, inflammatory status, methionine cycle and glycolysis. The most significantly changes in all studied groups connected with the metabolic pathway of Cysteine and methionine metabolism.

Keywords: COVID-19, cytokine storm, sepsis, targeted metabolomic analysis, LC-MS/MS, metabolic pathways

Abbreviations: CS, cytokine storm; ARDS, acute respiratory distress syndrome; SS, surgical sepsis; PCR, polymerase chain reaction; CCI, Charlson Comorbidity Index; SOFA, Sequential Organ Failure Assessment; ICU, intensive care unit; MES, 2-(N-morpholino)ethanesulfonic acid hydrate; QA/QC, quality control; RSD, relative standard deviation; SAH, S-

Adenosylhomocysteine; SAM, S-Adenosylmethionine; GABA, Gamma-Aminobutyric acid; TCA, Tricarboxylic acid cycle

1. Introduction

According to data as of January 14, 2024, the past SARS-CoV-2 pandemic claimed more than 7 million lives [1]. Despite a significant number of publications on COVID-19 (391381 in PubMed as of January 29, 2024), many questions related to the pathophysiology of the disease remain unresolved.

One extensively discussed syndrome linked to the illness is the cytokine storm (CS), observed in a substantial number of COVID-19 patients. CS, a systemic inflammatory state characterized by immune cell hyperactivation and uncontrolled cytokine release, is not exclusive to COVID-19. It is known that it can be triggered by various factors such as infections, tumor processes, autoimmune conditions, and more [2]. CS can precipitate acute respiratory distress syndrome (ARDS) or multiple organ dysfunction, which can potentially be fatal [3].

The clinical manifestations of COVID-19-associated CS and its consequences are similar to the manifestations of the acute phase of sepsis [4]. Sepsis, according to the Third International Consensus Definitions Task Force (Sepsis-3), is a life-threatening organ dysfunction caused by dysregulation of the host's response to infection [5]. Traditionally, bacterial infection was considered the major cause of sepsis [6]. The COVID-19 pandemic has led to a reassessment of the role of viruses in the occurrence of sepsis because multi-organ dysfunction caused by CS in COVID-19 largely corresponds to the concept of Sepsis-3 and is currently considered as viral sepsis [7].

Sepsis caused by CS associated with COVID-19 exhibits distinctive characteristics, even though its clinical symptoms typically resemble those of bacterial sepsis. COVID-19 is distinguished by a less pronounced and more prolonged occurrence of systemic multi-organ inflammation [8, 9], an accelerated onset of ARDS, reduced levels of inflammatory markers like IL-6 [10], and an immune signature due to differences in response to bacterial and viral infection [4].

This study focuses on comparing targeted metabolic profiles in the blood serum of patients with surgical sepsis (SS) and those with CS associated with COVID-19. The clinical data of patients with CS do not fully meet the Sepsis-3 criteria due to the patients' blood being collected at an early stage of disease development (before treatment), when the rate of development of multiple organ dysfunction in this group is quite low yet (SOFA≤2). Variations in the SOFA scores are significant due to the late manifestation of multiple organ dysfunctions in COVID-19 patients, the opposite of the explosive course of the disease, which is a characteristic of bacterial sepsis [9]. Current hypotheses suggest a connection between CS, caused by COVID-19, and the occurrence of viral sepsis [11], and that's why the comparison of patients with COVID-19 associated with CS and patients with SS seems reasonable. However, detailed knowledge about the pathophysiology of severe cases of COVID-19 associated with CS and bacterial sepsis is still insufficient. Comparative metabolome studies may help identify the differential and common pathobiological characteristics of these syndromes and supplement the knowledge base.

Comorbidities can make the course of COVID-19 more severe and enhance CS [12, 13]. In this regard, it would be interesting to determine how comorbidity would affect the results of comparing the metabolomic profiles of patients with COVID-19 and septic patients.

This study is one of the first metabolomic studies using a biobank and a significant amount of samples from COVID-19 patients with cytokine storm and surgical sepsis from St. Petersburg and the Leningrad region (Russian Federation). The study was carried out on serum material collected strictly before the beginning of treatment, which made it possible to obtain fairly "clean" serum samples without additional drug interference in the patients' metabolome. The aim of this study was to compare the serum metabolomic profiles of patients with COVID-19-associated CS with the serum metabolomic profiles of septic patients after surgery.

2. Material and methods

2.1. Participants

Frozen blood serum was used from the collection of the biobank of the St. Petersburg State Healthcare Establishment "City Hospital No. 40". The study was conducted within the framework of the research project "Biobanking and biomedical research of human tissue and fluid samples" and was approved by the Expert Council on Ethics of the St. Petersburg State Healthcare Establishment "City Hospital No. 40" (session No. 119, February 9, 2017). A total of 234 patients who underwent treatment at City Hospital No. 40 took part in the current retrospective study of the metabolomics profile. Samples were collected during hospital stays from January 2018 to March 2021 for septic patients and from May 2020 to June 2021 for COVID-19 patients. All patients underwent standard examinations, according to clinical recommendations and according to diagnosis. Informed consent for sample collection and placing samples in a biobank for subsequent use for scientific purposes and for the results' publication was obtained from all patients. The study was conducted following the World Medical Association's Code of Ethics (Declaration of Helsinki) for experiments involving humans.

The patients were grouped as follows:

1. COVID(-): COVID-19 patients with CS without comorbidity (n=40).
2. COVID(+): COVID-19 patients with CS with comorbidity (n=43)
3. Sepsis: SS patients (n=41).
4. Control: healthy volunteers (n=110).

COVID-19 was diagnosed by a polymerase chain reaction (PCR) of nasopharyngeal swabs. CS was determined according to the following conditions: ferritin > 485 μg/L, C-reactive protein > 50 mg/L, d-dimer > 2.5 μg/mL, interleukin-6 > 25 pg/mL, LDH > 550 U/L.

Comorbidities were determined based on the patient's self-report on admission and confirmed, if necessary, by further examinations. The Charlton Comorbidity Index (CCI) was calculated for each patient with COVID-19 [14]. Patients with CCI≤2 were included in the COVID(-) group, and patients with CCI≥5 were included in the COVID(+) group.

The diagnosis of sepsis was made according to the Sepsis-3 consensus criteria and the SOFA scale [5].

The Control group was chosen during regular preventive screenings. The criteria for inclusion in the group were the absence of COVID-19, confirmed with PCR, and the absence of sepsis. The general inclusion criterion was age over 18 years. Since our study is conducted in a real-world setting, we didn't employ any additional inclusion or exclusion criteria.

2.2. Study design

The objective of this study was to compare the serum metabolomic profiles of patients with CS associated with COVID-19 and patients with sepsis. Metabolomic profiles were obtained using target LC-MS/MS analyses. The study solved the following tasks:

1) t-SNE clustering of patients.
2) Comparison of serum metabolomes of patients of the COVID(-) and COVID(+) groups: identification of common and differentially represented metabolites that alter levels relative to the Control group.
3) Identification of metabolites differentially represented in the serum of the Sepsis group compared to the Control.
4) Comparison of serum metabolomic profiles of patients of the Sepsis groups with serum metabolomes of patients in the COVID(-) and COVID(+) groups: identification of common and differentially represented metabolites.

2.3. Sample collection and storage

Blood samples from patients diagnosed with COVID-19 were collected within day of hospital admission and prior to treatment initiation. Blood samples from septic patients were collected when they were admitted to the intensive care unit (ICU) before starting antibiotic treatment. The blood samples for the Control group were obtained during a routine examination of volunteers. All samples were collected into Vacutest tubes (Gel and clot act., Vacutest Kima S.R.L., Italy). After centrifugation for 10 min at 4^0C, 2,200 rpm, the serum was collected and immediately frozen at -80^0C. All samples were annotated, indicating the stage of the disease, gender, age, etc. Before analysis, the frozen samples were slowly warmed to room temperature and thoroughly mixed.

2.4. Metabolomic profiling of serum samples

The preparation of all blood serum samples for targeted metabolome studies was carried out in duplicate. 2-(N-morpholino)ethanesulfonic acid hydrate (MES) (CAS: 4432-31-9; cat no. M8250, Sigma-Aldrich) and L-methionine sulfone (CAS: 7314-32-1, cat No. M0876, Sigma-Aldrich) were used as internal standards. The samples were thawed at room temperature, and 100 µl of an ice-cold mixture of internal standards of a fixed concentration in acetonitrile (cat no. 9012.2500GL, LC-MS-grade, J.T. Baker) was added to 50 µl of serum. The mixture was vortexes and incubated for 10 min, then centrifuged (Centrifuge 5810R, Eppendorf) at 12000 g for 10 min at 4 °C. An aliquot of 80 uL supernatant was diluted with Milli-Q - water acidified with formic acid (cat No. 533002, for LC-MS LiChropur, >99%, Merck) to pH 2 and analyzed using the LC-MS/MS method. The prepared standard solutions and extracts were stored at -20 ^{0}C.

Targeted metabolite profiling was performed using a liquid chromatography-mass spectrometer with a triple quadrupole LCMS-8050 (Shimadzu) along with a Nexera X2 (Shimadzu) chromatography system. The analysis was carried out following the established "LC/MS/MS Method Package for Primary Metabolites" by Shimadzu, utilizing the multiple reaction monitoring mode. This method allows simultaneous analysis of 98 analytes of the main chemical classes of clinically significant low-molecular compounds, including amino acids, organic acids, nucleotides, nucleosides, and coenzymes (Table S1, S2). An analytical column

Discovery HS F5-3 (150 x 2.1 mm, 3 mkm) (Supelco, Merck) and SecurityGuard “SupelGuard Discovery HS F5-3” (20 x 2.1 mm, 3 mkm, Supelco) were used to separate the analytes. Mass-spectrometry parameters and chromatographic conditions were meticulously set in accordance with the guidelines provided in the manual for the "LC/MS/MS Method Package for Primary Metabolites" method. Data collection and processing were performed using LabSolutions software.

Metabolites were identified based on chromatographic retention time, m/z values of daughter ions, and their intensity ratios. Only chromatographic peaks with a peak-to-noise cutoff ratio ≥ 10 were considered. The content of the investigated substances was determined using the internal standard method, which considers the response (area) of the analyte connections in relation to the response (area) of the internal standard. All level measurements are given in arbitrary units of the content of the internal standard (arbitrary units — a.u.). The results correspond to the average of two parallel measurements of the same sample.

To ensure quality control (QA/QC), quality controls were analyzed along with experimental samples to monitor instrument performance and facilitate chromatographic alignment. Control samples consisted of extracts of averaged blood serum samples with internal standards, prepared following the same procedure as the experimental samples. The averaged serum, derived from thoroughly mixed blood serum samples from seven donors, was aliquoted in 200 µl portions and frozen at -80 °C for later analysis. An appropriate volume of Milli-Q water passed through all stages of sample preparation was used as a “blank” sample. Additionally, all solvents used in sample preparation were also analyzed.

We also utilized quality controls to assess the reproducibility and stability of the prepared extracts on both intra- and inter-day bases. The standard solutions and control extracts were examined over a 10-day period, when they were stored at -20 °C between analyses and once at +5 °C for 24 hours. The discrepancy between parallel measurements remained within 15% of the average values, with average daily deviations below 20%. The scatter of the obtained values was evaluated during averaging, ensuring a maximum difference of 20% between the averaged parallel measurements. If this threshold was exceeded, the sample was reanalyzed, and the initial result was disregarded.

Instrument variability was determined by calculating the median relative standard deviation (RSD) of the internal standards added to each sample before injection into the mass spectrometers. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrumental standards) present in 100% of the pooled matrix samples. Quality control samples were examined after every 50-60 test sample injections in order to ensure the consistency of the chromatographic retention times and responses of the studied compounds.

2.5. Statistical analysis

To test the hypothesis of normal data distribution, the Shapiro-Wilk test was used. Data were transformed using Median & Quantile Absolute Deviation based Z-Score. To identify intergroup differences in the concentration levels of the studied metabolites, assessed through a.u., a nonparametric one-way analysis of variance was performed using the Kruskal-Wallis test; the Mann-Whitney test was used as a post-hoc analysis. The data was clustered using the t-distributed stochastic neighbor embedding (t-SNE) method, and the resulting data was visualized in two-dimensional space.

The log fold change (lfc) was used as a measure reflecting the difference in the range of values between samples; descriptive statistics are presented by the median (Me) and interquartile range [Q1-Q3]. The difference between samples was considered significant at $p<0.05$ and $|lfc|>0.5$. A Volcano plot was used to visualize the test results. Data processing and statistical analysis were performed using the R programming language version 4.3.1 and the Python programming language version 3.12. Metabolic pathway analysis was performed using the MetaboAnalyst 6.0 package.

3. Results

3.1. Patient characteristics

Table 1
Patient demographic and clinical characteristics.

Characteristic	COVID(-) (n=40)	COVID(+) (n=43)	Sepsis (n=41)	Control (n=110)
Age, y	51±9.8	74±9	65.2±16.1	48.1±14.5
Sex (Male), n	24	23	19	77
CCI	1.5 ± 0.7	7.7 ± 3	-	-
SOFA	2 ± 0.5	1.5 ± 1.2	7.3 ± 3.6	-
Leukocytes cells, 10^9/L	7.9 ± 4.3	8.6 ± 4.8	16.8 ± 10.8	-
Neutrophils, 10^9/L	5.9 ± 3.6	7.1 ± 4.6	13.7 ± 9.3	-
Lymphocytes, 10^9/L	1.4 ± 0.7	1 ± 0.5	0.8 ± 0.4	-
IL-6, pg/mL	258.2 ± 742.7	346.6 ± 763.3	-	-
Creatinine, µmol/L	117.6 ± 106.3	112.8 ± 61.5	213.8 ± 149.8	

CCI - Charlson Comorbidity Index; SOFA - Sequential Organ Failure Assessment; "-" - data not available

Table 1 presents the demographic and clinical characteristics of the patient groups included in the study. The average age of comorbid patients was slightly higher than the average age in other groups (mean 74±9 y vs. 51±9.8 y for COVID(-), 65.2±16 y for Sepsis and 48.1±14 y for Control). The imbalance can be explained by the fact that comorbidities usually arise at a later age.

3.2. Clustering of the patients

The levels of 100 compounds were studied using the LC/MS/MS procedure (Table S1, S2). 83 compounds whose results were differed from zero were taken for the further analysis (Table S3).

t-SNE clustering of patients was performed based on the obtained metabolomic data (Table S3). It revealed 3 clear clusters groups: Sepsis, Control and COVID-19 (Fig. 1). Interestingly, the COVID(-) and COVID(+) groups formed approximately one cluster,

practically not separated. The Control group was notably segregated, situated far away from the other clusters. Additionally, data from sepsis patients was also well separated from others, while being in proximity to a cluster of COVID-19 patients.

3.3. COVID(-) vs. COVID(+) metabolomics

For detalization, we compared the metabolomic profiles of COVID(-) and COVID(+) patients (Table S3, Fig. 2). 62 and 67 metabolites were changed compared to the Control in COVID(-) and COVID(+) groups ($p<0.05$), respectively. A decline of 38 metabolites along with rise of 24 compounds was recorded in COVID(-) serum; the most prominent changes are presented in Figure 2A. While for COVID(+) group, 45 metabolites showed a decrease and 22 metabolites exhibited an increase compared to the Control group (Figure 2B). Among 9 compounds mostly increased in the COVID(-) group, 6 metabolites were also risen in the COVID(+) group. These were dimethylglycine, L-acetylcarnitine, L-kynurenine, L-phenylalanine, L-cystathionine, adenosine monophosphate (Fig. 2 AB). Among 25 metabolites mostly decreased in the COVID(+) group, the levels of 17 compounds also fell in the COVID(-) group. These were L-histidine, citrulline, ornithine, uridine, uric acid, L-arginine, asymmetric dimethylarginine, pantothenic acid, L-threonine, 4-hydroxyproline, choline, allantoin, inosine, glycine, L-leucine, acetylcholine chloride, L-isoleucine (Fig. 2 AB).

In spite of the presence of some common characteristics of metabolomics changes, there were 29 compounds, which revealed significant ($p<0.05$) differences in the levels between the COVID(-) and COVID(+) groups (Table S3, Fig 2 C). 15 metabolites exhibited higher levels in the COVID(+) group compared to the COVID(-) group. L-kynurenine, L-lactic acid, L-alanine, uric acid, uracil, carnosine, ornithine, and norepinephrine were the most prominent. Conversely, 14 metabolites showed decreased levels in the COVID(+) group, with L-proline and serine being the most notable (Fig. 2 C). Interestingly, some of these compounds changed their levels in series from Control to COVID(-) and then to COVID(+) group. For example, L-kynurenine and ornithine revealed such dynamics (Table S3 for full data).

To evaluate the potential metabolomic shifts that resulted from changes in measured compounds, an enriched pathway analysis was held. We identified the top 8 common pathways from the KEGG database that were most significantly dysregulated in the COVID(-) and COVID(+) groups compared with the Control group (Fig. 3 AB; Table S4 AB). The four of these pathways were uniquely disturbed in each group (Fig. 3 C; Table S4 D). The metabolism of cysteine and methionine showed the largest difference, being more disrupted in COVID(+) vs. COVID(-) patients (Fig. 3 AB; Table S4 AB).

These data, in consistency with t-SNE clustering (p. 3.2), demonstrated that the difference between COVID(+) and COVID(-) groups was not substantial and had rather quantitative than qualitative character.

3.4. Sepsis metabolomics

In comparing the Sepsis and Control groups, we found differences in the levels of 75 metabolites ($p<0.05$) (Table S3, Fig. 4 A). Among these metabolites, 35 exhibited elevated levels and 40 metabolites displayed decreased levels. The most prominent changes were displayed on Figure 4 A. Enriched pathway analysis identified the top 10 pathways in the KEGG database that were potentially disturbed in the Sepsis group (Fig. 4 B; Table S4 C). These pathways included

arginine biosynthesis; cysteine and methionine metabolism; alanine, aspartate, and glutamate metabolism; glycine, serine, and threonine metabolism; citrate (TCA) cycle, arginine and proline metabolism; pyrimidine metabolism; tyrosine metabolism; histidine metabolism, and glutathione metabolism.

3.5. Sepsis vs. COVID-19 metabolomics

Comparison of the Control group with the Sepsis, COVID(-), and COVID(+) groups indicated significant differences in metabolite abundance ($p \leq 0.01$). Among the groups, the greatest number of changes in metabolite levels (72) was observed in the Sepsis vs. Control comparison. In the comparison of COVID(-) vs. Control, 59 metabolites showed varying levels, while 61 metabolites displayed differences in the COVID(+) vs. Control comparison (Table S3, Fig. 6, 7).

The levels of 50 metabolites changed significantly ($p \leq 0.01$) both for COVID(-) and Sepsis groups relative to the Control group. Within these groups, the levels of 11 metabolites increased, and 22 metabolites decreased. Similarly, the levels of 54 metabolites showed significant changes in both the COVID(+) and Sepsis groups relative to the Control group; the levels of 14 metabolites increased and 25 metabolites decreased in both groups (Table S3). All three groups exhibited increased levels of 11 metabolites relative to the Control. The most prominent were dimethylglycine, L-acetylcarnitine, L-cystathionine, adenosine monophosphate. Additionally, 19 metabolites displayed lower levels compared to the controls across all groups. The most prominent are L-histidine, citrulline, ornithine, uridine, pantothenic acid, L-threonine, choline, inosine, L-leucine, and L-isoleucine (Fig. 2 AB, 4 A).

Comparison of the metabolomic profiles of the Sepsis and COVID(-) groups showed that the levels of 60 metabolites were significantly different ($p<0.05$) (Table S3). Among these, the levels of 38 metabolites showed higher levels in the Sepsis group, and 22 metabolites displayed lower levels in the Sepsis group. The most significant changes were displayed on Figure 6 A.

The comparison of metabolomic profiles between the Sepsis and COVID(+) groups showed that the levels of 56 metabolites were significantly different ($p<0.05$) (Table S3). Of these, in the Sepsis group, the levels of 36 metabolites were higher and the levels of 20 metabolites were lower. The most significant changes were displayed on Figure 7 A.

The levels of 36 metabolites were significantly different ($p \leq 0.01$) in both COVID-19 groups relative to the Sepsis group; of these, the levels of 28 metabolites were higher in the serum of septic patients (Table S3, Fig. 6 A, 7 A). The most prominent were acetylcholine chloride, L-histidine, uric acid, allantoin, 4-hydroxyproline, asymmetric dimethylarginine, creatine, creatinine, citric acid, methionine sulfoxide, guanosine, L-carnitine, L-cystathionine, carnosine. The levels of 12 compounds were lower in the serum of septic patients compared to COVID-19 one. The most prominent were L-proline, L-aspartic acid, L-tryptophan, niacinamide, L-tyrosine, L-phenylalanine, L-glutamic acid (Fig. 6 A, 7 A).

Some metabolites levels changed solely in the Sepsis or COVID-19 groups. So as 4-hydroxyproline, isocitric and pyruvic acids, procollagen-5-hydroxylisine, creatine, creatnine, SAM, acetylcholine chlorid, cytric acid, serotonine, symmetric dimethiylarginine significantly increased, and L-aspartic acid, L-glutamic acid, L-proline, L-lysine, GABA, niacinamide significantly decreased exclusively in the Sepsis group. In contrast to the Sepsis group, both groups of patients with COVID-19 were characterized by a significant increase in citicoloine, 5-thymidilic acid, GABA, nicotinic acid, L-phenylalanine, histamine and a decrease in allantoin, 4-

hydroxiproline, isocytric and pyruvic acids, procollagen-5-hydroxylisine, acetylcholine chloride, symmetric and asymmetric dimethylarginine, L-methionine, uric acid, and L-lactic acid (Table S3).

Enriched pathway analysis of the KEGG databases identified eight metabolic pathways, the differences in which for septic and COVID-19 (COVID(-) and COVID(+)) patients were most pronounced ($p \leq 0.05$) (Fig. 6 B, 7 B; Table S4 EF). These pathways remained consistent when comparing sepsis with both COVID(-) and COVID(+). There were cysteine and methionine metabolism, histidine metabolism, arginine and proline metabolism, arginine biosynthesis pathway, aspartate, glutamate, and alanine metabolism, phenylalanine, tyrosine, and tryptophan biosynthesis pathways, phenylalanine metabolism, and pyrimidine metabolism.

4. Discussion

We compared the serum metabolome of patients with COVID-19 related CS vs. surgical sepsis. Both syndromes stem from infections, with COVID-19 linked to a viral infection and surgical sepsis to a bacterial one. These conditions could lead to an increased inflammatory response, posing notable risks to the patients involved. We carried out this study to improve understanding of the pathophysiology that underlies these hyperinflammatory processes.

The presence of comorbidities can make the course of COVID-19 more severe [12, 13]. To examine the impact of concomitant illnesses on metabolomic alterations and their relevance in relation to the Sepsis group, two patient groups with CS were formed: one without comorbidities (COVID(-)) and the other presenting comorbidities (COVID(+)). The metabolomic profiles of these groups were also compared.

Our research uncovered parallel shifts in the metabolome both the COVID(+) and COVID(-) group. The levels of almost all amino acids, including proteogenic and glycogenic types, as well as crucial markers of energy metabolism like pyruvate, lactate, and TCA cycle acids, experienced a significant decrease (Table S3). Other researchers have also reported a reduction in the levels of these metabolites in patients with CS [15, 16]. Furthermore, it was shown that there was a link between the reduction in amino acid levels and the increasing of cytokines in the plasma [17]. In this study, patients experienced more significant changes in certain energy-related metabolites within the COVID(+) group, where the IL6 level was elevated (Table 1). Both COVID-19 groups were characterized by decreased carnitine along with increased acetylcarnitine levels, which might indicate an energy deficiency and impaired transport in mitochondria. Similar changes were found by other authors [18, 19, 20]. Also a reduction in the levels of arginine, citrulline, and ornithine indicates a significant disturbance in nitrogen metabolism in COVID-19 patients, aligning with findings from other researchers [21, 22]. The pathway of tryptophan degradation, linked to inflammation modulation, underwent a common alteration in both groups of COVID-19, shifting towards kynurenine synthesis. These results in elevated kynurenine levels and decreased levels of tryptophan and serotonin are consistent with the existing data [18]. In COVID-19 patients, elevated levels of some other metabolites linked to the inflammatory response were observed. These included the inflammatory mediator histamine and the neurotransmitter gamma-aminobutyric acid (GABA), known to possess anti-inflammatory properties [23]. We assumed that the increase in GABA levels was compensatory. The disparities between the COVID(-) and COVID(+) groups can essentially be considered as inconsequential. The most significant variations in the dynamics of changes were observed with L-proline and serine. Their levels sharply rose in the COVID(-) group and approached the control level in the COVID(+) group. It could be supposed that the

alterations in these amino acids in patients with comorbidities were linked to the features of accompanying illnesses and their therapies. So we can propose that the main changes in metabolomics profiles in both groups of COVID-19 patients were due to the virus infection, not comorbidities. This proposal is aligned with the conclusion in [24].

The obtained data on the metabolomics of septic patients, presented in Table S3 and Fig. 3A, were consistent with the data of other researchers. This change in the level of proteogenic and glycogenic amino acids was considered one of the characteristic features of sepsis [25]. The drop in the level of serum amino acids was explained by their use as a substrate for the TCA cycle and glycolysis for the energy demand sharply increasing during sepsis [26]. Energy imbalance in the Sepsis group was confirmed in our study by increased levels of oxoglutaric and citric acids, metabolites of the TCA cycle.

Another feature of sepsis is a violation of beta-oxidation of fatty acids in mitochondria and an increase in the level of acetylcarnitines [27]. In our study, L-acetylcarnitin was elevated in septic patients. Also, septic patients showed elevated kynurenine levels and declined in tryptophan and serotonin levels, suggesting a redirection of tryptophan degradation toward kynurenine synthesis, as indicated in Table S3. These findings aligned with the data presented in [27]. The inflammatory marker procollagen 5-hydroxy-L-lysine was also significantly increased in the Sepsis group. Similar results were obtained in the study [28]. The rise in dopamine and acetylcholine chloride levels could be attributed to the compensatory anti-inflammatory impact of these metabolites [29, 30]. In addition, we detected a significant change in the levels of compounds in septic patients associated with the development of oxidative stress - decreased levels of glutathione and increased levels of its precursor gamma-glutamylcysteine, ophthalmic acid and symmetric dimethylarginine, noted by other authors [26, 31, 32]. Overall, the metabolomic profile data of patients with COVID-19 and Sepsis aligned with findings from other researchers.

We observed a similarity in the direction of level changes for various metabolites when comparing the metabolomic profiles of patients with COVID-19 and sepsis, across the Control - COVID(-) - COVID(+) - Sepsis series (Table S3, Fig. 2 AB, Fig. 4 A). For example, the levels of L-alanine, ornithine, and uric acid sequentially increased. It could be linked to higher breakdown of proteins and nucleic acids caused by cell death and inflammation due to a more severe course of pathological process in the COVID(+) group than in the COVID(-) group and more inflammation and oxidative stress in the Sepsis group than in COVID-19 patients [26, 33]. An increase in uric acid levels was also a marker of gradual deterioration of renal function in the series Control - COVID(-) - COVID(+) - Sepsis [34]. This was confirmed by clinical analysis of creatinine levels of patients (Table 1). The inflammatory marker kynurenine showed elevated levels across all three groups. Surprisingly, in the Sepsis group, its level was the lowest, while in the groups of patients with COVID-19, its level was notably higher, with the COVID(+) group displaying the highest level. Another participant in the tryptophan degradation pathway, serotonin, exhibited the opposite alteration. Its level was highest in the Sepsis group, and lowest in the COVID(+) group. This fact required additional examination to comprehend the involvement of the kynurenine pathway in hyperinflammatory conditions of diverse origins. The level of the antioxidant carnosine sequentially increased in the series COVID(-) - COVID(+) - Sepsis. Moreover, its level in the COVID(-) group was lower than the level in the Control group, but significantly higher in the COVID(+) and Sepsis groups. Also in the series of COVID(-), COVID(+), and Sepsis, a consistent rise in levels was noted for norepinephrine, a metabolite known for its reported anti-inflammatory properties [35]. At the same time, the levels of the

precursor of norepinephrine in the synthesis pathway, L-tyrosine, altered in opposite way and fell proportionally in the series. This suggests that there could be compensatory production of carnosine and norepinephrine as inflammation and oxidative stress escalated within the sequence Control - COVID(-) - COVID(+) - Sepsis.

The sequential increase in lactic acid in the series COVID(-) - COVID(+) - Sepsis looked explicable. As lactic acid levels rose in the examined groups, it indicated a transition towards glycolysis, signaling a shift in energy metabolism. In all three groups, it was noteworthy that the level of lactic acid remained lower compared to the Control group, despite documented elevations in lactic acid levels observed in sepsis and severe cases of COVID-19 [36, 37].

Considerable alterations in all groups were found for the methionine metabolism. In the progression from COVID(-) to COVID(+) to Sepsis, a notable alteration in the concentrations of two metabolites associated with the methionine cycle was detected: a rise in SAH levels and a decline in serine levels. It should be noted that a decrease in serine levels could impact both methionine synthesis and the pathway for producing the antioxidant glutathione, which exhibited similarly low levels across all three groups. In the progression from COVID(-) to COVID(+) to Sepsis, the methionine sulfoxide, another participant in the methionine cycle, showed a steady rise in levels. We assumed that this was necessary to maintain the level of methionine, as a key metabolite in many processes, when the level of serine fell.

Levels of several metabolites fell to a similar extent in patients with COVID-19 and in the Sepsis group (Table S3, Fig. 2 AB, Fig. 4 A). Firstly, it is the decrease in amino acid levels observed in both the Sepsis group and patients with COVID-19. This level drop might indicate a heightened demand for oxidative sources to energy cycles across all groups [16]. Levels of glutathione and choline, known for their ability to decrease oxidative stress and inflammation, diminished equally in individuals with sepsis and with COVID-19.

So, while there were resemblances in metabolomic profiles, the distinctions in them for sepsis and COVID-19 patients were also significant. Fig. 5 schematically demonstrated the number of metabolites that significantly changed their levels in the three groups. The greatest number of such metabolites belonged to the Sepsis group (72); the COVID(-) and COVID(+) groups differed slightly in this feature (59 and 61 metabolites, respectively), but the difference between both COVID-19 groups and Sepsis group was a lot more. It might be explained by the different dynamics of both pathological processes. As noted, sepsis characterizes by more "explosive" course than CSS, which also leads to severe consequences, but more slowly [33].

For the intergroup comparison of Sepsis vs. COVID-19, 28 metabolites showed a significant increase ($p \leq 0.01$) compared to both groups of COVID-19 patients (p. 3.4). These included metabolites related to mitochondrial function, markers of oxidative stress, collagen distruction, methionine and transsulfuration cycles, and renal failure. The most prominent were differences in the levels of metabolites in the TCA cycle. It likely reflected the biological basis of both syndromes. COVID-19 causes, in most cases, lung injury at the beginning of the infection and oxygen deficiency as a consequence. So the suppression of the TCA cycle occurred. Surgical sepsis is not necessary related to the hard lung injury [33], especially at the early stage, as for our patients. In the absence of hypooxigenation, the TCA cycle upregulates in order to satisfy the increased needs in energy production for maintenance of hypperinflamation and immune function.

Some metabolites exhibited a significant ($p \leq 0.01$) downregulation in septic patients compared to COVID-19 patients (Fig. 6 A, 7 A, Table S3). The lower levels of several amino acids in the Sepsis group might be associated with the greatest need for energy and more

intensive utilization of amino acids in energetic cycles. But the most significant changes were noted in the profiles of GABA and niacinamide. These metabolites were increased in the COVID(-) and COVID(+) groups, but their levels decreased by numerous orders of magnitude in the Sepsis group. GABA might reveal an anti-inflammatory function [23], and niacinamide was the precursor in synthesis of NAD. Such alterations might indicate a significantly higher level of inflammation and energy problems in septic patients compared to patients with COVID-19.

To evaluate the potential changes in the metabolomics pathway, we carried out an enriched analysis of the KEGG databases. The analysis revealed that certain metabolic pathways shared among the COVID(-), COVID(+), and Sepsis groups were notably disrupted. These pathways included: glycine, serine and threonine metabolism; arginine metabolism; cysteine and methionine metabolism; arginine and proline metabolism; alanine, aspartate and glutamate metabolism; glutathione metabolism; tyrosine metabolism; and histidine metabolism (Fig. 3 AB, Fig. 4 B; Table S4). Among these, the cysteine and methionine metabolism pathway was one of the most altered in all groups (Fig. 3 C, Fig. 6B, Fig. 7 B). By assessing the level of changes in this pathway based on the number of metabolites analyzed, the *p*-value, and the impact factor, one could see greater impairment in the COVID(+) and sepsis groups than in the COVID(-) group. The cysteine and methionine metabolism pathway is one of the most important in the body. It is associated both with maintaining the level of methionine, which is a donor of methyl groups, and with the transformations of sulfur-containing amino acids responsible for the redox potential. In our study, the arginine biosynthesis and histidine metabolism pathways were also altered similarly in all three groups. The TCA cycle was disturbed only in the COVID(+) and Sepsis groups, but to a greater extent in the Sepsis group as assessed by the number of analyzed metabolites, p-value and impact factor. This analysis confirmed that significant metabolic changes in the serum of COVID-19 and septic patients were primarily linked to amino acid metabolism and alterations in redox potential and energy cycle, with metabolic irregularities amplifying from COVID(-) to COVID(+) to Sepsis.

This study possessed several limitations. Firstly, we carried out targeted metabolomic exploration, so our results did not cover metabolomic changes as completely as a non-targeted metabolomic study can. Secondly, there was a substantial disparity in SOFA levels between the patient groups of COVID-19 and Sepsis (Table 1). This incongruity arose from our deliberate collection of blood samples from COVID-19 patients prior to treatment initiation to mitigate the impact of antibiotics, glucocorticoids, and similar factors. So, the observed SOFA discrepancy appeared due to the slower progression of multiple organ failure in COVID-19 compared to bacterial sepsis. Lastly, our study reflected real-world conditions, resulting in variations in age parameters across the groups under comparison (Table 1). It is imperative to consider these limitations when evaluating the findings of this research.

5. Conclusion

This study used biobank samples of COVID-19 patients with cytokine storm and surgical sepsis from St. Petersburg and the Leningrad region (Russian Federation) and represented one of the first serum comparative metabolomic studies in this geographical area. The serum of patients with COVID-19 and sepsis showed significant changes in various metabolites linked to amino acid metabolism, nitrogen metabolism, inflammation, the folate and methionine cycles, and glycolysis. Differences between COVID(-) and COVID(+) groups were not significant. Changes in metabolite levels tended to increase consistently from COVID(-) to COVID(+) to Sepsis groups, with more pronounced changes in the Sepsis group. The most significant differences

between septic and COVID-19 patients appeared in metabolites related to kynurenine synthesis, niacinamide, the TCA cycle, and GABA. Across all groups, there were significant alterations in the cysteine and methionine metabolism pathways. So, our study revealed common and different features of the metabolomic profiles of patients with sepsis and CS associated with COVID-19.

Consent for publication

All authors agree to the publication of the manuscript.

Institutional review board statement

The study was conducted following the World Medical Association's Code of Ethics (Declaration of Helsinki) for experiments involving humans and was approved by the Expert Council on Ethics of the St. Petersburg State Healthcare Establishment "City Hospital No. 40" (session No. 119, February 9, 2017).

Informed consent statement

Written informed consent was obtained from all subjects involved in the study.

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CRediT authorship contribution statement

Russkikh Ia.V.: Investigation, Validation, Data curation, Writing – original draft, Writing – review & editing. Popov O.S.: Conceptualization, Investigation, Data curation, Formal analysis, Visualization, Writing – review & editing. Klochkova T.G.: Conceptualization, Writing – original draft, Writing – review & editing. Sushentseva N.N.: Conceptualization, Methodology, Writing – review & editing. Apalko S.V.: Conceptualization, Project administration, Writing – review & editing. Asinovskaya A.Yu.: Resources, Project administration, Supervision. Mosenko S.V.: Data curation, Resources. Sarana A.M.: Supervision, Project administration. Shcherbak S.G.: Resources, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All experimental details, results, and materials are available in the text and supplementary file. Clinical information involving the true

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None.

Supplementary data

Supplementary data includes baseline information on the list of studied compounds, MRM transitions used for the studied compounds, primary statistics data, pathway analysis tables.

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Captions for figures

Fig. 1. Differences in metabolomics between the COVID(-), COVID(+) and Control groups resulted from the t-SNE clustering of patients based on the LC-MS/MS analysis.

Fig. 2. Comparison of COVID(-), COVID(+) and Control groups. The volcano plots show most significant differentially abundant metabolites. Log2 fold change cutoff 0.5; *p*-value cutoff 0.05**. A.** COVID(-) vs. Control, metabolites highlighted in red were overexpressed in COVID(-). **B.** COVID(+) vs. Control, metabolites highlighted in red were overexpressed in COVID(+). **C.** COVID(-) vs. COVID(+), metabolites highlighted in red were overexpressed in COVID(-).

Fig. 3. The main KEGG pathways disturbed in groups. **A.** COVID(-) vs. Control. **B.** COVID(+) vs. Control. **C.** COVID(-) vs. COVID(+). Dots present the most relevant pathways. The pathway impact is presented by size and *p*-value by color.

Fig. 4. Comparison Sepsis and Control groups. **A.** Volcano plot Sepsis vs. Control. The plot shows most significant differentially abundant metabolites ($p \leq 0{,}01$). Log2 fold change cutoff 0.5, metabolites highlighted in red are overexpressed in Sepsis. **B.** The main KEGG pathways disturbed in Sepsis vs. Control. Dots present the most relevant pathways. The pathway impact is presented by size and *p*-value by color.

Fig. 5. Differentialy abundant metabolites in comparison with Control.

Fig. 6. Comparison Sepsis and COVID(-) groups. **A.** Volcano plot Sepsis vs. COVID(-). The plot shows most significant differentially abundant COVID(-) metabolites ($p \leq 0{,}01$). Log2 fold change cutoff 0.5, metabolites highlighted in red were overexpressed in Sepsis. B. The main KEGG pathways disturbed in Sepsis vs. COVID(-). Dots present the most relevant pathways. The pathway impact is presented by size and *p*-value by color.

Fig. 7. Comparison Sepsis and COVID(+) groups. A. Volcano plot Sepsis vs. COVID(+). The plot shows most significant differentially abundant COVID(-) metabolites ($p \leq 0{,}01$). Log2 fold change cutoff 0.5, metabolites highlighted in red were overexpressed in Sepsis. B. The main KEGG pathways disturbed in

Sepsis vs. COVID(+). Dots present the most relevant pathways. The pathway impact is presented by size and *p*-value by color.