ALTERED AMINO ACID PROFILES OF THE “MOTHER–FETUS” SYSTEM IN COVID-19

Lomova NA ✉, Chagovets VV, Dolgopolova EL, Novoselova AV, Petrova UL, Shmakov RG, Frankevich VE

Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology, Moscow, Russia

Systemic nature of the human body response to SARS-CoV-2 requires dedicated analysis at the molecular level. COVID-19 during pregnancy affects maternal health and may entail complications in the early neonatal period and possibly long-term consequences for the offspring. The aim of the study was to assess the impact of COVID-19 on amino acid profiles in maternal venous blood, amniotic fluid and umbilical cord blood in order to develop a diagnostic panel accounting for possible consequences. The main group included 29 pregnant patients with a confirmed diagnosis of COVID-19 and the control group included 17 somatically healthy pregnant women. Amino acid profiles of the biological fluids were measured by high-performance liquid chromatography combined to mass spectrometry (HPLC-MS) and assessed in logistic regression models. The analysis revealed altered content of certain amino acids, their biosynthetic precursors and metabolites in the biological fluids collected from patients with COVID-19 possibly reflecting the development of systemic inflammatory reaction and associated changes in gene expression profiles. These findings may guide further research into health outcomes for neonates born from mothers infected with SARS-CoV-2 during pregnancy. The study may help to develop advanced recommendations and differential care protocols for pregnant women and newborns diagnosed with COVID-19.

Keywords: COVID-19, amino acids, mass spectrometry, blood plasma, umbilical cord blood, venous blood, amniotic fluid, clinical neonatal markers, metabolic pathways

Funding: the study was funded by the Russian Foundation for Basic Research, grant reg. number 2004-60093.

Author contribution: Lomova NA — clinical data management, manuscript writing; Chagovets VV —mass spectrometry assay, statistical analysis of the data, manuscript editing; Dolgopolova EL — collection and processing of clinical samples in “red zone”, statistical analysis of the data; Novoselova AV — mass spectrometry assay, processing of spectral data; Petrova UL — collection and processing of clinical samples in “red zone”; Shmakov RG — clinical sample collection and data management, manuscript editing; Frankevich VE — concept, data management, writing and editing of the manuscript.

Compliance with ethical standards: the study was approved by Ethical Review Board at the V.I. Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology (protocol № 13 of 10 December 2020) and carried out in compliance with Declaration of Helsinki, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guideline, and Federal Law № 323-FZ “On Basics of Health Protection of Citizens in the Russian Federation” of 21 November 2011. Informed consent for the study was provided by all participants.

✉ **Correspondence should be addressed:** Natalia A. Lomova
Oparina, 4, Moscow, 117997, Russia; natasha-lomova@yandex.ru

Received: 14.04.2022 **Accepted:** 28.04.2022 **Published online:** 17.05.2022

DOI: 10.24075/brsmu.2022.025

ИЗМЕНЕНИЕ АМИНОКИСЛОТНОГО ПРОФИЛЯ В СИСТЕМЕ «МАТЬ–ПЛОД» ПРИ COVID-19

Н. А. Ломова ✉, В. В. Чаговец, Е. Л. Долгополова, А. В. Новоселова, У. Л. Петрова, Р. Г. Шмаков, В. Е. Франкевич

Национальный медицинский исследовательский центр акушерства, гинекологии и перинатологии имени В. И. Кулакова, Москва, Россия

Вирус SARS-CoV-2 оказывает значительное влияние на организм человека, и актуален вопрос о характере этого воздействия на молекулярном уровне. COVID-19 не только оказывает влияние на мать в период беременности, но и повышает риск осложнений в раннем неонатальном периоде и может иметь отдаленные последствия для здоровья новорожденного. Целью исследования было определить влияние COVID-19 на аминокислотный состав венозной крови беременных, амниотической жидкости и плазмы пуповинной крови для разработки диагностической панели, а также провести анализ возможных последствий для состояния новорожденного. Основную группу составили 29 пациенток с подтвержденным диагнозом COVID-19; контрольную группу — 17 соматически здоровых женщин. На первом этапе работы был проведен анализ аминокислотного профиля. Обнаруженные различия в содержании аминокислот в различных биологических жидкостях позволили разработать модели логистической регрессии. По данным математического анализа задействованности метаболических путей маркеров-аминокислот в венозной и пуповинной плазме крови матерей и новорожденных в группе с COVID-19 обнаружено статистически значимое изменение биосинтеза и путей метаболизма ряда аминокислот, задействованных в реализации воспалительной реакции, изменений энергетического метаболизма, нарушений регуляции экспрессии и транскрипции белковых молекул и пр. Эти результаты могут быть использованы для выбора направления дальнейших исследований возможных последствий для здоровья новорожденных от матерей, перенесших COVID-19, и определения требований к лечению и медицинской помощи беременным женщинам и новорожденным после постановки диагноза COVID-19.

Ключевые слова: COVID-19, аминокислоты, масс-спектрометрия, плазма, пуповинная кровь, венозная кровь, амниотическая жидкость, маркеры состояния новорожденного, метаболические пути

Финансирование: работа выполнена при финансовой поддержке РФФИ грант рег. № 2004-60093.

Вклад авторов: Н. А. Ломова — анализ клинических данных, систематический анализ, написание рукописи; В. В. Чаговец — проведение метаболомного анализа методом масс-спектрометрии, статистический анализ полученных данных, редактирование рукописи; Е. Л. Долгополова — сбор и подготовка биологических сред в условиях «красной зоны», статистический анализ результатов; А. В. Новоселова — проведение метаболомного анализа методом масс-спектрометрии, обработка масс-спектрометрических данных; У. Л. Петрова — сбор и подготовка биологических сред в условиях «красной зоны»; Р. Г. Шмаков — анализ клинических данных в условиях «красной зоны», систематический анализ, редактирование рукописи; В. Е. Франкевич — подготовка исследования, систематический анализ, написание и редактирование рукописи.

Соблюдение этических стандартов: исследование одобрено этическим комитетом НМИЦ АГП им. В. И. Кулакова (протокол № 13 от 10 декабря 2020 г.), проведено в соответствии с требованиями Хельсинкской декларации, Международной конференции по гармонизации (ICF), Стандартов надлежащей клинической практики (GCP), ФЗ «Об основах охраны здоровья граждан в Российской Федерации» № 323-ФЗ от 21 ноября 2011 г. Все пациентки подписали добровольное информированное согласие на участие в исследовании.

✉ **Для корреспонденции:** Наталья Анатольевна Ломова
ул. Академика Опарина, д. 4, г. Москва, 117997, Россия; natasha-lomova@yandex.ru

Статья получена: 14.04.2022 **Статья принята к печати:** 28.04.2022 **Опубликована онлайн:** 17.05.2022

DOI: 10.24075/vrgmu.2022.025

The past 50 years encountered over 300 outbreaks of new or long-forgotten infections, including three coronavirus infections in 2002, 2012, and 2019. These viral outbreaks, ignited by direct contacts between old and new host species of the virus, reflect the lack of compliance with sanitary and hygienic requirements typical for Asian marketplaces. Coronaviruses make up an extensive family of 40 viruses, seven of which have been implicated in human diseases. Their emergence results from the continuous biological evolution: certain viral strains that prosper in animals gradually evolve the capability to infect humans. The infamous connection between SARS-CoV-2 and humanity was likely initiated at a large animal and seafood wholesale market in Wuhan. The severe acute respiratory syndrome outbreak caused by this virus in China rapidly escalated to global pandemic challenging healthcare systems with the unpreceded demand for intensive care.

A number of studies indicate higher severity of COVID-19 in pregnant women compared to age-matched non-pregnant controls [1]. In addition, COVID-19 during pregnancy has been associated with increased risks of premature labor [1, 2]. Advanced maternal age, excessive body weight, and comorbidities such as hypertension or diabetes increase the risks of COVID-19 pregnancy complications [3].

The isolated reported cases of vertical transmission of the virus, as well as serious maternal complications in the antenatal period might need advanced verification [4–7]. According to the WHO, all studied samples of amniotic fluid and breast milk were SARS-CoV-2-negative. The ability of the active virus to be transmitted from mother to fetus or newborn during pregnancy and childbirth has not been characterized thoroughly. At the same time, newborns from SARS-CoV-2 infected mothers may be at higher risks of early neonatal complications and long-term health consequences.

Omics studies, metabolomics in particular, represent a promising direction of medical research. The high-throughput approaches help to identify candidate markers for secure diagnostics, patient management optimization and prognosis. A 2020 study correlated 204 metabolites in blood plasma of patients with the severity of COVID-19 [8]. Amino acids, an integral part of the metabolome, are pivotal markers of physiological status. Optimal levels of amino acids in the body ensure metabolic equilibrium and smooth functioning of organ systems, whereas amino acid imbalances indicate the opposite. Altered amino acid concentrations have been observed in cardiovascular disease [9], H1N1 influenza virus-associated pneumonia [10] and chronic obstructive pulmonary disease [11]. Certain neonatal pathologies, notably the storage diseases, can be diagnosed by measuring the amino acid content in dried blood spots [12]. The disease-related changes of metabolic profiles in biological fluids of the human body provide valuable diagnostic substratum for a number of pathological conditions. For example, altered amino acid profiles in patients with COVID-19 have been associated with compromised oxygen homeostasis [13]. Another study on amino acid profiles in adults and children with COVID-19 identified changes plausibly associated with endothelial and T cell dysfunctions [14, 15].

In our previously published article, we searched for COVID-19 predictive markers in amniotic fluid and cord blood [16]. In the current study, we analyze amino acid composition of maternal venous blood, amniotic fluid and umbilical cord blood as metabolomic projection of the “mother–fetus” system in order to provide a relevant description of affected metabolic pathways and account for potential clinical significance and long-term consequences of COVID-19 in pregnancy.

METHODS

Patient enrollment and clinical data and sample collection were carried out in March–May 2020 at the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V. I. Kulakov, its First Infectious Unit refurbished into “red zone” for patients diagnosed with COVID-19 including those pregnant.

The study enrolled 46 pregnant women admitted for observation and delivery at the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V. I. Kulakov. The main group included 29 patients with confirmed diagnosis of COVID-19 and the control group included 17 somatically healthy women without pregnancy complications. The diagnosis of COVID-19 was verified by PCR test (DNA Technology LLC; Russia). The enrollment was carried out on recourse. Inclusion in the main group was based on positive PCR test for COVID-19. Inclusion in the control group was based on the lack of clinical symptoms and negative PCR test for COVID-19. Exclusion criteria for the study encompassed multifetal pregnancy, rhesus- and AB0-isoimmunizations, as well as chromosome aberrations, genetic mutations and congenital malformations in the fetus.

Samples of venous blood plasma from pregnant participants, umbilical cord blood plasma from their newborns and amniotic fluid were collected for the analysis. Sample transportation was carried out within the premises of the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov for subsequent analysis in lab spaces certified for biosafety level II. We measured 31 amino acids in physiological fluids by high-performance liquid chromatography-combined mass spectrometry (HPLC-MS) using standard kits and a protocol from the manufacturer (JASEM; Turkey). The samples were analyzed in a 1260 Infinity II LC system (Agilent; USA) with MS detection in a 6460 Triple Quad instrument (Agilent; USA). The transitions from parent ions to daughter fragments for the analyzed amino acids, corresponding chromatographic retention times, internal standard concentrations, as well as sensitivity and reproducibility parameters for the analysis are described in the JASEM manual.

Statistical analysis of the data

Statistical processing of the data was carried out using R scripts (R Core Team; Austria) in RStudio integrated environment (RStudio, Inc.; USA). The normality of quantitative data distributions was assessed by Shapiro–Wilk test. Normal distributions were described by means and standard deviations. Distributions other than normal were described by medians with lower and upper quartiles in the Me (Q1; Q3) format. The comparisons involved parametric Student’s t-test for the data complying with normal distributions and nonparametric Mann–Whitney test for distributions other than normal. The threshold p-value was accepted 0.05; p-values smaller than 0.001 were underspecified as $p < 0.001$. The feasibility of patient stratification of the basis of amino acid profiles was assessed by logistic regression method. The initial set of logistic regression models were built using all possible combinations of amino acids as independent variables possibly reflecting affiliation of the patient with particular group of the study (dependent variable). These first-round models were subjected to receiver operating characteristic (ROC) analysis and candidate models with the highest area-under-the-curve (AUC) values were selected for subsequent validation. Each model was characterized

Table 1. Clinical characterization of the main group (patients with COVID-19 while pregnant)

Clinical characteristics	COVID-19, *n* = 29 (%)
Clearly symptomatic	21 (71.41)
Elevated body temperature (> 37 °C)	12 (41.38)
Anosmia	7 (24.14)
Sore throat	3 (10.34)
Dyspnea	4 (13.79)
Cough	12 (41.38)
Fatigue	4 (13.79)
Mild symptoms	22 (75.86)
Moderate symptoms	6 (20.69)
Severe symptoms	1 (3.45)

by Wald's test with corresponding 95% confidence interval (CI) and odds ratio (OR) with CI. The quality assessment for the developed models involved determination of sensitivity and specificity by ROC analysis. Metabolomic implications of the identified between-the-group differences were assessed using MetaboAnalyst version 5.0 tool (https://www.metaboanalyst.ca/) connected to the KEGG compound and pathway database (Kyoto Encyclopedia of Genes and Genomes). Statistical significance for a metabolic pathway influence was determined by hypergeometric test controlled with Benjamini–Hochberg procedure. The assessment of individual marker contributions involved topological analysis and relative mediation effect. Associations with the disease were considered significant at false discovery rates below the level of significance (FDR < 0.05).

RESULTS

The study enrolled 46 pregnant participants: 29 inpatients with confirmed diagnosis of COVID-19 (main group) and 17 conditionally healthy inpatients (control group). Clinical characterization for the main group (patients with COVID-19 while pregnant) is given in Table 1; clinical characterization for the entire cohort (both groups) is given in Table 2.

None of the newborns had COVID-19. All newborns were tested for SARS-CoV-2 immediately after birth, and repeatedly on days 3 and 10 of life. The negative test results may indicate the lack of vertical transmission. No perinatal deaths occurred in the studied cohort.

The laboratory assay used commercial reagent kits for targeted quantitative measurement of 31 amino acids in biological samples of maternal venous blood plasma, amniotic fluid and umbilical cord blood plasma by HPLC-MS.

At the first stage of the analysis we measured amino acid content of the maternal venous blood plasma for the two groups of the study. Statistical analysis of HPLC-MS data identified five amino acids with concentrations differing significantly between the groups: 1-methylhistidine, lysine, cystine, glutamic acid and glutamine (Table S1, Fig. 1).

The identified differences inspired the search for a mathematical model that would enable distinguishing between SARS-CoV-2-positive pregnant patients and matched controls on the basis of amino acid content of the venous blood plasma. The corresponding HPLC-MS measurements were used as a basis for logistic regression models. The first round of modeling employed all possible combinations of amino acids. All developed models were subject to ROC analysis and four models with the highest AUC values were selected for subsequent validation (Fig. S1, Table S2).

The maximal AUC of 0.78 was shown by a model involving methylhistidine and cystine, characterized by 0.93 sensitivity and 0.94 specificity (Table S3).

A similar HPLC-MS profiling of 31 amino acids in the amniotic fluid and statistical analysis of the data revealed eight amino acids with concentrations differing significantly between the groups: 1-methylhistidine, 3-methylhistidine, arginine, cystathionine, cystine, glutamine, histidine and trans-4-hydroxyproline (Fig. 2, Table S4).

A search for the means of metabolomic differentiation between SARS-CoV-2-positive pregnant patients and matched controls was subsequently carried out for the amniotic fluid samples; the results are given in Fig. S2 and Tables S5 and S6. Construction of logistic regression models for amino acid profiles in amniotic fluid followed the same algorithm as for the venous blood plasma. In this series, the maximal AUC of 0.89 was shown by a model that involved arginine, cystine, histidine and trans-4-hydroxyproline, characterized by 0.84 sensitivity and 0.93 specificity (Table S6). Somewhat higher levels of sensitivity and specificity (0.84 and 1, respectively) were shown by a model that involved 1-methylhistidine, cysteine and trans-4-hydroxyproline (Table S6).

Table 2. Clinical characterization of the two groups of the study

Parameter	COVID-19, *n* = 29	Control group, *n* = 17	*p*-value
Age	29.9 (± 5.03)	32.0 (± 5.03)	0.16
Height	166.62 (± 7.37)	165.76 (± 7.34)	0.71
Weight	77.64 (± 11.58)	71.87 (± 9.75)	0.10
BMI	27.85 (± 4.52)	26.12 (± 3.16)	0.18
Term at delivery	38 (± 1.52)	39.42 (± 1.14)	0.001
Weight at birth	3332 (± 484)	3585 (± 424)	0.08
Height at birth	52.4 (± 2.66)	53.1 (± 2.29)	0.35
Apgar score at 1 min	8 (8; 8)	9 (9; 9)	0.69
Apgar score at 5 min	8 (8; 8)	9 (9; 9)	0.83

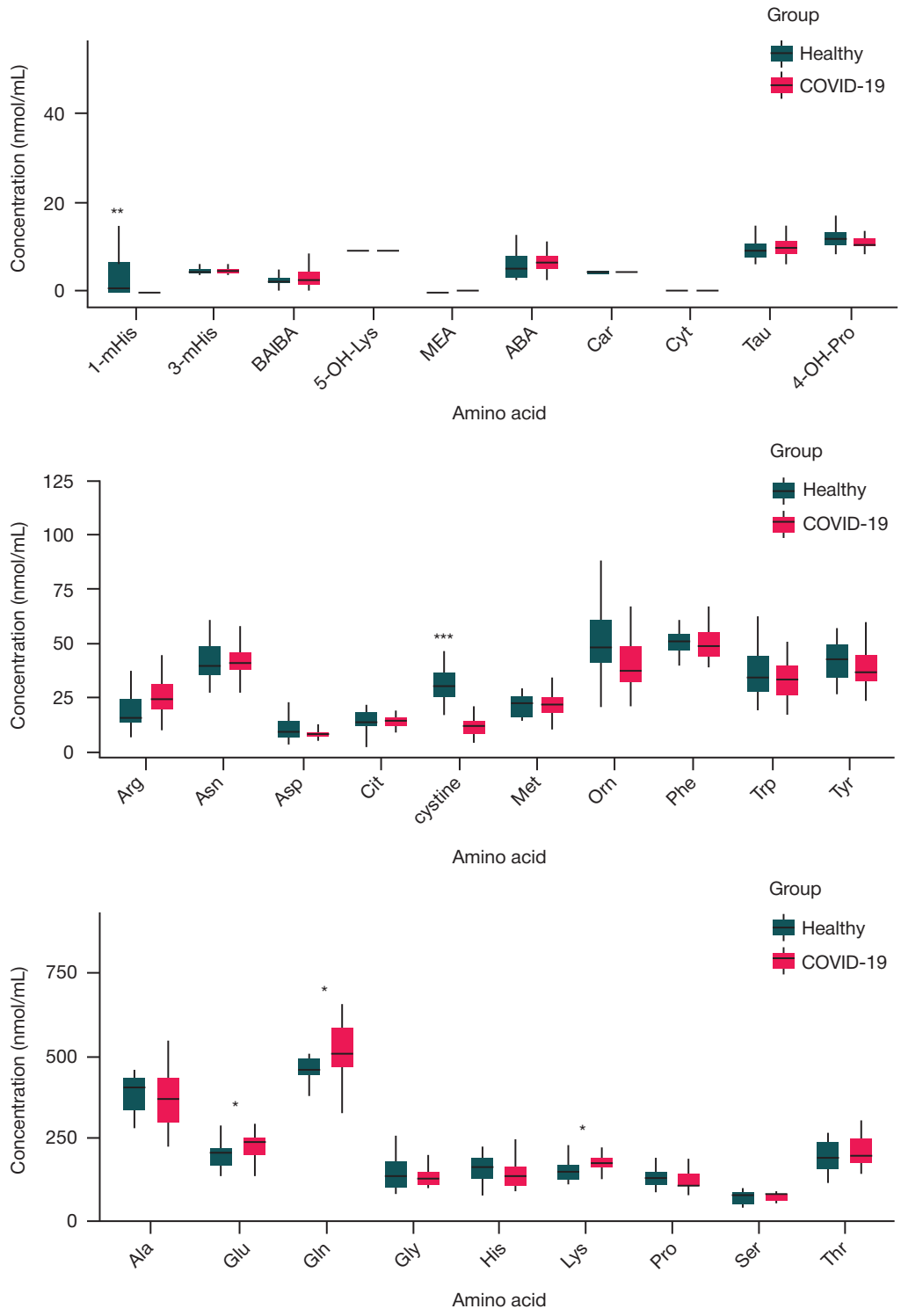

Fig. 1. Boxplots of amino acid concentrations in the maternal venous blood plasma for the two groups of the study. Box limits represent lower and upper quartiles (respectively, Q1 and Q3); box heights are interquartile ranges (IQR); horizontal lines in the boxes are medians (Q2); the whisker termini are within Q3+1.5 IQR (upper) and Q1-1.5 IQR (lower); * — *p*-value ≤ 0.05; ** — *p*-value ≤ 0.01; *** — *p*-value ≤ 0.001. 1-mHis — 1-methyl-L-histidine; 3-mHis — 3-methyl-L-histidine; bAla — β-alanine; BAIBA — 3-aminoisobutyric acid; 5-OH-Lys — DL-5-hydroxylysine; MEA — ethanolamine; ABA — L-2-aminobutyric acid; AAD — L-2-aminoadipic acid; Ala — L-alanine; Arg — L-arginine; Asn — L-asparagine; Asp — L-aspartic acid; Car — L-carnosine; Cit — L-citrulline; Cyt — L-cystathionine; cystine — L-cystine; Glu — L-glutamic acid; Gln — L-glutamine; Gly — L-glycine; His — L-histidine; Lys — L-lysine; Met — L-methionine; Orn — L-ornithine; Phe — L-phenylalanine; Pro — L-proline; Ser — L-serine; Thr — L-threonine; Trp — L- tryptophan; Tyr — L-tyrosine; Val — L-valine; Tau — taurine; 4-OH-Pro — *trans*-4-hydroxy-L-proline

A similar analytical scheme was applied to the umbilical cord blood plasma samples. Statistical analysis of the spectral data revealed four candidate amino acids with concentrations differing significantly between the groups: 1-methylhistidine, β-alanine, cysteine and histidine (Fig. 3, Table S7).

Construction of logistic regression models for amino acid profiles of the umbilical cord blood plasma was carried out similarly with other sample types; the results are given in Fig. S3 and Tables S8 and S9. All successful regression models built in this series involved cystine, which showed the most pronounced between-the-group differences. All models obtained in this series had AUC = 1 at sensitivity and specificity of 1 (Table S9).

The three types of studied biological samples (amniotic fluid, maternal venous blood and umbilical cord blood plasma)

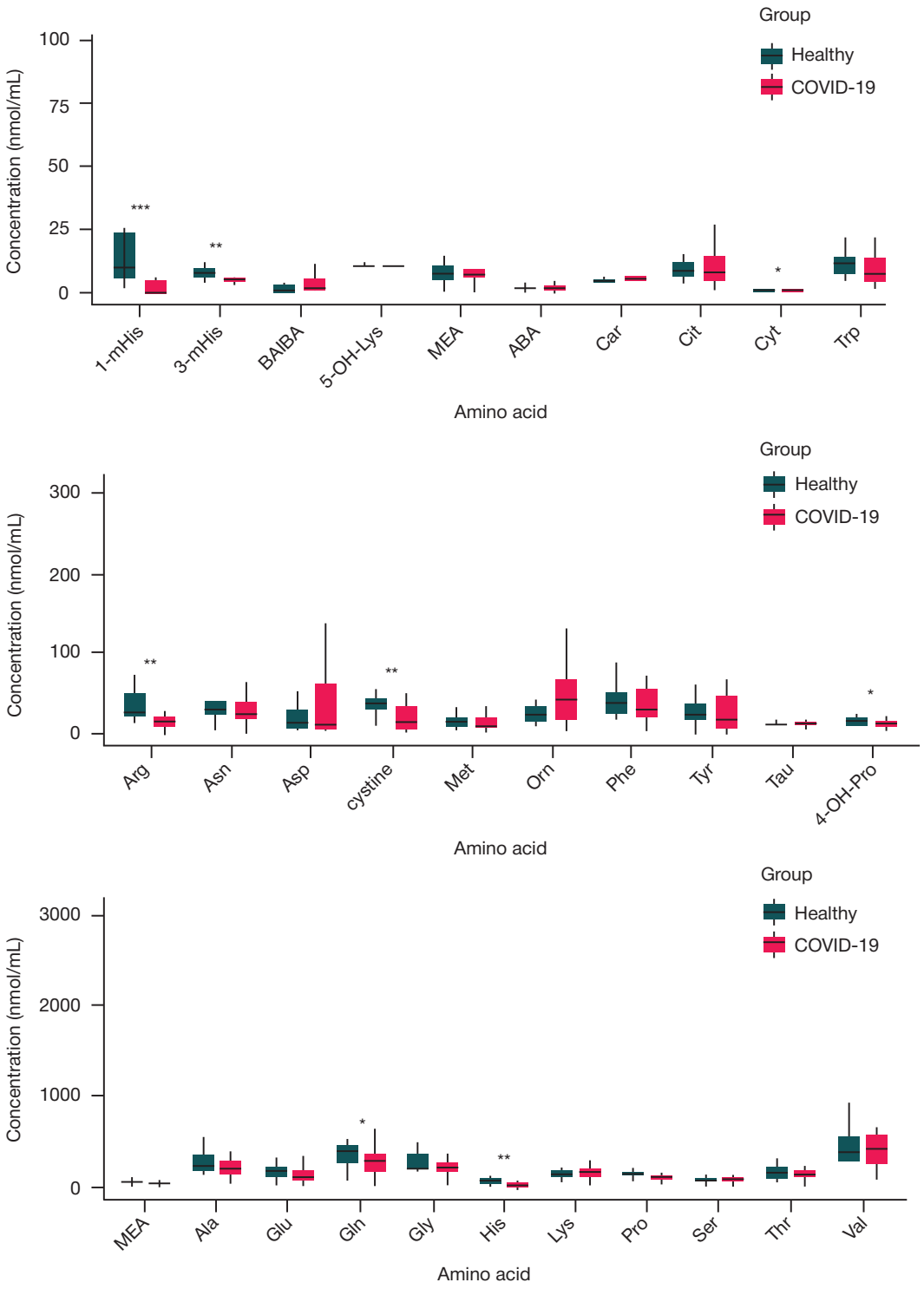

Fig. 2. Boxplots of amino acid concentrations in the amniotic fluid for the two groups of the study. Box limits represent lower and upper quartiles (respectively, Q1 and Q3); box heights are interquartile ranges (IQR); horizontal lines in the boxes are medians (Q2); the whisker termini are within Q3+1.5 IQR (upper) and Q1-1.5 IQR (lower); * — p-value ≤ 0.05; ** — p-value ≤ 0.01; *** — p-value ≤ 0.001. 1-mHis — 1-methyl-L-histidine; 3-mHis — 3-methyl-L-histidine; BAIBA — 3-aminoisobutyric acid; 5-OH-Lys — DL-5-hydroxylysine; MEA — ethanolamine; ABA — L-2-aminobutyric acid; AAD — L-2-aminoadipic acid; Car — L-carnosine; Cit — L-citrulline; Cyt — L-cystathionine; 4-OH-Pro — *trans*-4-hydroxy-L-proline

had certain matches among the amino acids with significant between-the-group concentration differences. Concentrations of 1-methylhistidine and cystine showed COVID-19-related differences in all three types of biological samples thus most consistently reflecting the metabolome of the mother–fetus system. L-Histidine, a coded amino acid and precursor of 1-methylhistidine, showed differential content in both maternal and fetal plasma, but not in amniotic fluid. Another marker, glutamine, 'responded' to COVID-19 in maternal venous blood plasma and amniotic fluid, but not in umbilical cord blood plasma (Table S10).

The identification of recurring statistically significant changes for the content of certain amino acids in three biological media of the mother–fetus complex during SARS-CoV-2 infections required advanced interpretation. In this regard, we analyzed participation of these amino acids in key metabolic pathways and their possible clinical significance for the mother and the fetus, including potential long-term consequences. The

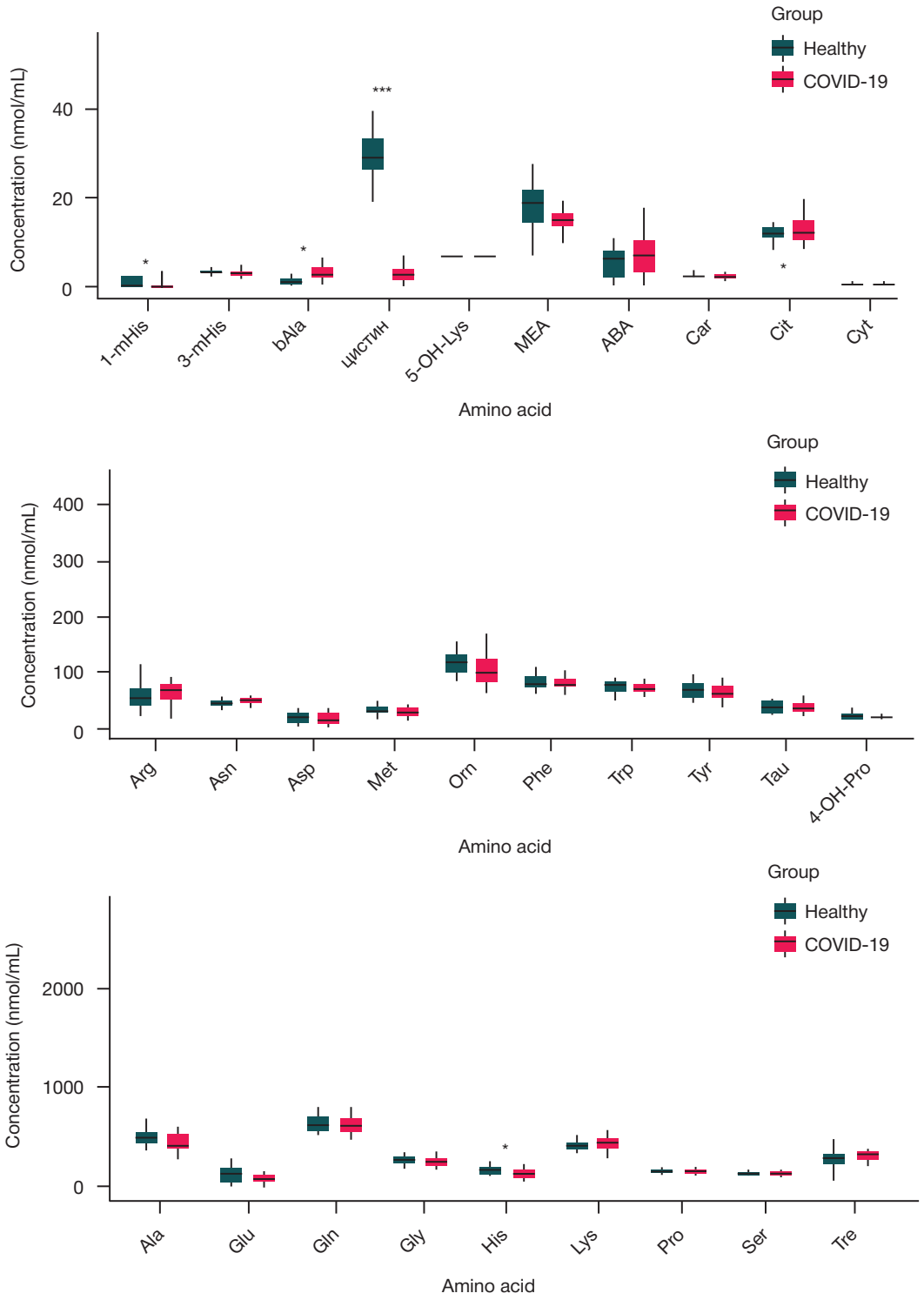

Fig. 3. Boxplots of amino acid concentrations in the umbilical cord blood plasma for the two groups of the study. Box limits represent lower and upper quartiles (respectively, Q1 and Q3); box heights are interquartile ranges (IQR); horizontal lines in the boxes are medians (Q2); the whisker termini are within Q3+1.5 IQR (upper) and Q1-1.5 IQR (lower); * — *p*-value ≤ 0.05; ** — *p*-value ≤ 0.01; *** — *p*-value ≤ 0.001. 1-mHis — 1-methyl-L-histidine; 3-mHis — 3-methyl-L-histidine; bAla — β-alanine; BAIBA — 3-aminoisobutyric acid; 5-OH-Lys — DL-5-hydroxylysine; MEA — ethanolamine; ABA — L-2-aminobutyric acid; AAD — L-2-aminoadipic acid; Ala — L-alanine; Arg — L-arginine; Asn — L-asparagine; Asp — L-aspartic acid; Car — L-carnosine; Cit — L-citrulline; Cyt — L-cystathionine; 4-OH-Pro — *trans*-4-hydroxy-L-proline

analysis employed MetaboAnalyst version 5.0 tool (https://www.metaboanalyst.ca/) connected to the KEGG database (Kyoto Encyclopedia of Genes and Genomes).

For amniotic fluid, the identified COVID-19-associated changes in amino acid profiles revealed no significant matches with specific metabolic pathways (Fig. 4A, Table S11). For umbilical cord blood plasma, the observed changes implicated histidine and β-alanine metabolism imbalances (Fig. 4B, Table S12). For maternal venous blood plasma, the plausibly affected pathways included D-glutamine and D-glutamate metabolism, arginine biosynthesis and alanine, aspartate and glutamate metabolism (Fig. 4C, Table S13).

DISCUSSION

The obtained results indicate significant differences in concentrations of certain amino acids in biological fluids of pregnant patients with and without COVID-19, including eight

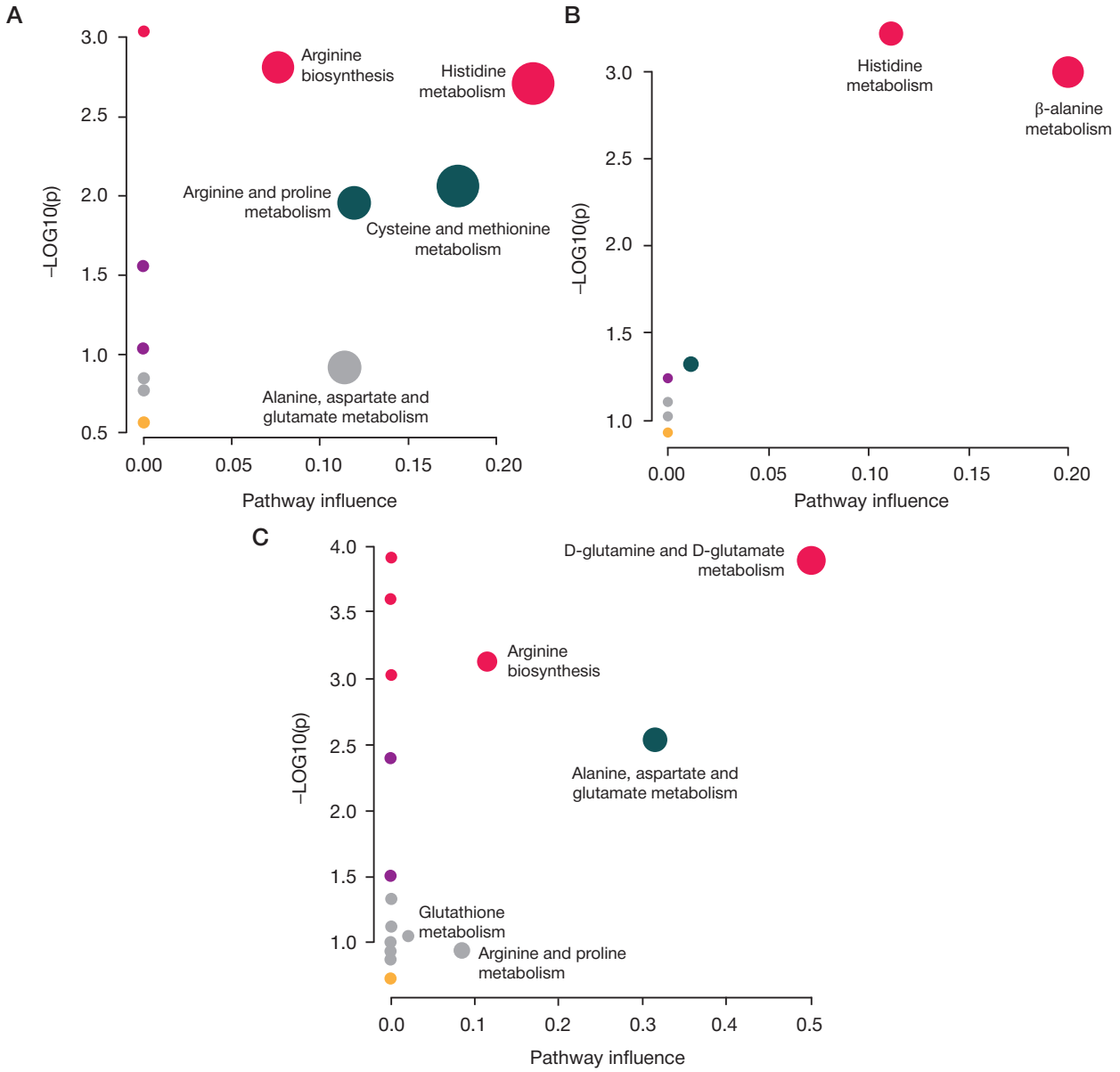

Fig. 4. Charts of the metabolic pathway influence for amino acid markers in the studied biological samples: amniotic fluid (**A**); cord blood plasma (**B**); venous blood plasma (maternal, **C**)

amino acids in amniotic fluid, five amino acids in maternal venous blood plasma and four amino acids in umbilical cord blood plasma. Of those, eight amino acids, notably arginine, had reduced concentrations in the biological fluids of patients with COVID-19. Similar changes in plasma levels of arginine and its metabolites in COVID-19 observed by other authors [14] may reflect endothelial dysfunction implicated in the development of COVID-19 lung injury [17, 18]. Low bioavailability of arginine has been associated with dysregulated endothelial and T cell functionalities [19, 20] among other pathophysiological consequences [21]. Apart from the reduced arginine levels, patients with COVID-19 presented with significantly altered systemic concentrations of citrulline, glutamine, alanine, glycine, histidine, proline and several other amino acids, though the mechanisms and pathophysiological interpretation of these changes are elusive [14]. Reduced systemic levels of amino acids are typical for other pathological conditions as well [9, 11, 12, 14, 22, 23]. In our setting, COVID-19-related changes in amino acid profiles of the maternal venous blood plasma revealed statistically supported associations with D-glutamine and D-glutamate metabolism, as well as alanine, aspartate and glutamate metabolism and notably the arginine biosynthesis pathways.

The semi-essential or conditionally essential amino acid arginine is one of the most metabolically versatile amino acids serving as a precursor for the synthesis of urea, nitric oxide, polyamines, proline, glutamate, creatine and agmatine. Arginine is metabolized via a complex and tightly regulated set of pathways which remain understudied at both cellular and systemic levels. A decrease in arginine concentration resulting from altered arginase activity may selectively affect expression of specific genes [24]. The arginine deficiency-associated abnormal protein expression in cell cultures has been studied for over 40 years [25]. Arginine is a prominent small-molecule regulator of gene expression [26–29].

Amino acids showing synchronous concentration dynamics in amniotic fluid, umbilical cord blood and maternal venous blood in ‘response’ to COVID-19 are particularly interesting. Significant concentration differences in all three studied biological media were revealed for two amino acids, 1-methylhistidine and cystine, with particularly high differentiating significance demonstrated for the latter — a non-coded amino acid derived

from oxidative dimerization of cysteine. L-Cystine represents a key biochemical module in creation and maintenance of the tertiary structure of proteins and peptides, indispensable for their biological activity. For example, peptide hormones vasopressin, oxytocin, insulin and somatostatin acquire their biological activity through formation of intramolecular disulfide bridges.

Synchronous COVID-19-associated dynamics in maternal and fetal blood plasma was observed for L-histidine (a substrate of protein synthesis and precursor for 1-methylhistidine).

The altered content of amino acid markers observed by us in umbilical cord blood plasma implicated histidine and β-alanine metabolism imbalances.

The naturally occurring beta-amino acid β-alanine is formed through dihydrouracil and carnosine degradation. Carnosyl β-alanine and related dipeptides represent a water-soluble component of the cellular anti-oxidant protection system, complementing the fat-soluble membrane-anchored anti-oxidative agents. These dipeptides alleviate toxic effects of reactive oxygen species and unsaturated aldehydes through prevention of protein cross-linking [30]. The reported proliferative effect of β-alanine and related molecules is apparently confined to muscle and nervous tissues, characterized by low proliferative capacity and extremely strong oxidative metabolism. It can be assumed that, apart from the anti-oxidant activity in muscle tissue, these amino acids may contribute to regenerative response and stimulate proliferation of normal (non-tumor) human cells.

The implication of amino acid markers in histidine metabolism may link COVID-19 with disruption of histidine decarboxylation and histamine production pathways during pregnancy.

A coding heterocyclic α-amino acid L-histidine is one of the two conditionally essential amino acids (along with arginine), initially considered essential in children only. Histidine residues with their unique electronic properties are encountered in catalytically active centers of many enzymes. Histidine is an important precursor in histamine biosynthesis known to play a central role in inflammatory response and certain allergic reactions. Histidine is known to facilitate tissue growth and repair, nerve fiber myelination and hematopoiesis. Histidine deficiency has been shown to promote hearing loss and neurodegeneration.

Overall, the obtained results indicate that SARS-CoV-2 infections may have significant metabolomic effects reflected by altered profiles of marker amino acids in amniotic fluid and umbilical cord blood, as well as in maternal circulation. Considering the vital significance of proteinogenic amino acids, these alterations may affect protein production machinery at all levels of the mother–fetus system even in the absence of COVID-19 symptoms during parturition. Long-term effects of intrauterine exposure to COVID-19 remain a high priority clinical issue. Assessment of metabolic profiles in relation to viral replication, host inflammatory response and altered energy metabolism lays the foundation for future research in this direction.

CONCLUSIONS

In this study, we used the targeted metabolomics approaches to identify changes in systemic levels of amino acids in pregnant patients infected with SARS-CoV-2 at the time of admission to inpatient facilities. Statistically significant differences in concentrations between the control group of conditionally healthy pregnant women and the matched group of patients with COVID-19 were revealed for eight amino acids in the amniotic fluid (1-methylhistidine, 3-methylhistidine, arginine, cystathionine, cystine, glutamine, histidine and trans-4-hydroxiproline), five amino acids in the maternal venous blood plasma (1-methylhistidine, lysine, cystine, glutamic acid and glutamine) and four amino acids in the umbilical cord blood plasma (1-methylhistidine, β-alanine, cystine and histidine). Metabolic disorders involving the identified amino acids have been described in a number of pathological conditions including acute respiratory distress syndrome in severe sepsis, H1N1 influenza-associated pneumonia, bacterial pneumonia, sickle cell anemia, thalassemia, malaria, acute asthma, cystic fibrosis, pulmonary hypertension, cardiovascular diseases and certain cancers. Analysis of amino acid markers in representative biological fluids of the mother–fetus system revealed significant COVID-19-associated changes in the biosynthesis and catabolism of amino acids involved in inflammatory reactions and altered metabolic patterns with corresponding changes in gene and protein expression. These results may help investigate health outcomes of COVID-19 in newborns and their mothers, which may include endocrine, nervous, allergic and other components, in order to adjust clinical recommendations for COVID-19 in pregnancy.

References

1. Chiu-Lin Wang, Yi-Yin liu, Chin-Hu Wu, Chun-Yu Wang, Chun-Hung Wang, Cheng-Yu Long. Impact of COVID-19 on Pregnancy. International Journal of Medical Sciences. 2021; 18 (3): 763–7. DOI: 10.7150/ijms.49923.
2. Schwartz DA, Graham AL. Potential Maternal and Infant Outcomes from (Wuhan) Coronavirus 2019-nCoV Infecting Pregnant Women: Lessons from SARS, MERS, and Other Human Coronavirus Infections. Viruses. 2020; 12 (4): 194.
3. Zaigham M, Andersson O. Maternal and perinatal outcomes with COVID–19:A systematic review of 108 pregnancies. Acta Obstet Gynecol Scand. 2020; 99 (7): 823–9.
4. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet. 2020; 395 (10226): 809–15.
5. Sukhikh G, Petrova U, Prikhodko A, Starodubtseva N, Chingin K, Chen H, et al. Vertical Transmission of SARS-CoV-2 in Second Trimester Associated with Severe Neonatal Pathology. Viruses. 2021; 13 (3). DOI:10.3390/v13030447.
6. Ellington S, Strid P, Tong VT, Woodworth K, Galang RR, Zambrano LD, et al. Characteristics of women of reproductive age with laboratory-confirmed SARS-COV-2 infection by pregnancy status — United States, January 22-June 7, 2020. Morb Mortal Wkly Rep. 2020; 69 (25): 769–5.
7. Vivanti AJ, Vauloup-Fellous C, Prevot S, Zupan V, Suffee C, Do Cao J, et al. Transplacental transmission of SARS-CoV-2 infection. Nat Commun. 2020; 11 (1). DOI: 10.1038/s41467020-17436-6.
8. Shen B, Yi X, Sun Y, Bi X, Du J, Zhang C, et al. Proteomic and Metabolomic Characterization of COVID-19 Patient Sera. Cell. 2020; 182 (1): 59–72.
9. McGarrah RW, Crown SB, Zhang G, Shah SH, M.H.S., Newgard CB. Cardiovascular Metabolomics. Circ Res. 2018; 122 (9): 1238–58. DOI: 10.1161/CIRCRESAHA.117.311002.
10. Banoei MM, Vogel HJ, Weljie AM, Kumar A, Yende S, Angus DC, et al. Plasma metabolomics for the diagnosis and prognosis of

H1N1 influenza pneumonia. 2017; 1–15.
11. Inoue S, Ikeda H. Differences in plasma amino acid levels in patients with and without bacterial infection during the early stage of acute exacerbation of COPD. Int J COPD. 2019; 14: 575–83.
12. Moat SJ, George RS, Carling RS. Use of Dried Blood Spot Specimens to Monitor Patients with Inherited Metabolic Disorders. Int J Neonatal Screen. 2020; 6 (2): 1–17.
13. Páez-Franco JC, Torres-Ruiz J, Sosa-Hernández VA, Cervantes-Díaz R, Romero-Ramírez S, Pérez-Fragoso A, et al. Metabolomics analysis reveals a modified amino acid metabolism that correlates with altered oxygen homeostasis in COVID-19 patients. Sci Rep. 2021; 11 (1). DOI: 10.1038/s41598-021-85788-0.
14. Rees CA, Rostad CA, Mantus G, Anderson EJ, Chahroudi A, Jaggi P. Altered amino acid profile in patients with SARS-CoV-2 infection. 2021; 118 (25): 4–6.
15. Hirschel J, Vogel M, Baber R, Garten A, Beuchel C, Dietz Y, et al. Relation of whole blood amino acid and acylcarnitine metabolome to age, sex, BMI, puberty, and metabolic markers in children and adolescents. Metabolites. 2020; 10 (4). DOI: 10.3390/metabo10040149.
16. Lomova NA, Chagovets VV, Dolgopolova EL, Novoselova AV, Petrova UL, Shmakov RG, et al. Changes in amino acid profile of cord blood plasma and amniotic fluid of mothers with COVID-19. Bulletin of Russian State Medical University. 2021; (3): 12–22. DOI: 10.24075/BRSMU.2021.032
17. Diorio C, McNerney KO, Lambert M, Paessler M, Anderson EM, Henrickson SE, et al. Evidence of thrombotic microangiopathy in children with SARS-CoV-2 across the spectrum of clinical presentations. Blood Adv. 2020; 4 (23): 6051–63.
18. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nature Reviews Immunology. 2020; 20 (6): 363–74.
19. Gambardella J, Khondkar W, Morelli MB, Wang X, Santulli G, Trimarco V. Arginine and endothelial function. Biomedicines. 2020; 8 (8): 277.
20. Rodríguez PC, Ochoa AC. Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: Mechanisms and therapeutic perspectives. Immunological Reviews. 2008; 222 (1): 180–91.
21. Morris CR, Hamilton-Reeves J, Martindale RG, Sarav M, Ochoa Gautier JB. Acquired Amino Acid Deficiencies: A Focus on Arginine and Glutamine. In: Nutrition in Clinical Practice. SAGE Publications Inc., 2017; 30S–47S.
22. IKEDA H. Plasma amino acid levels in individuals with bacterial pneumonia and healthy controls. 2020; 1–17.
23. Ware LB, Magarik JA, Wickersham N, Cunningham G, Rice TW, Christman BW, et al. Low plasma citrulline levels are associated with acute respiratory distress syndrome in patients with severe sepsis. Crit Care. 2013; 17 (1): 1–8.
24. Morris SM Jr. Arginine: beyond protein. Am J Clin Nutr. 2006; 83: 508S–12S.
25. Schimke RT. Repression of enzymes of arginine biosynthesis in mammalian tissue culture. Biochim Biophys Acta. 1962; 62: 599–601.
26. Jackson MJ, Allen SJ, Beaudet AL, O'Brien WE. Metabolite regulation of argininosuccinate synthetase in cultured human cells. J Biol Chem. 1988; 263: 16388–94.
27. Lee J, Ryu H, Ferrante RJ, Morris SM Jr, Ratan RR. Translational control of inducible nitric oxide synthase expression by arginine can explain the arginine paradox. Proc Natl Acad Sci USA. 2003; 100: 4843–8.
28. Taheri F, Ochoa JB, Faghiri Z, Culotta K, Park HJ, Lan MS, et al. L-Arginine regulates the expression of the T-cell receptor zeta chain (CD3zeta) in Jurkat cells. Clin Cancer Res. 2001; 7: 958s–65s.
29. Fernandez J, Lopez AB, Wang C, Mishra R, Zhou L, Yaman I, et al. Transcriptional control of the arginine/lysine transporter, cat-1, by physiological stress. J Biol Chem. 2003; 278: 50000–9.
30. Cheng J, Wang F, Yu DF, Wu PF, Chen JG. The cytotoxic mechanism of malondialdehyde and protective effect of carnosine via protein cross-linking/mitochondrial dysfunction/reactive oxygen species/MAPK pathway in neurons. European Journal of Pharmacology. 2011; 650, 184–94.

Литература

1. Chiu-Lin Wang, Yi-Yin liu, Chin-Hu Wu, Chun-Yu Wang, Chun-Hung Wang, Cheng-Yu Long. Impact of COVID-19 on Pregnancy. International Journal of Medical Sciences. 2021; 18 (3): 763–7. DOI: 10.7150/ijms.49923.
2. Schwartz DA, Graham AL. Potential Maternal and Infant Outcomes from (Wuhan) Coronavirus 2019-nCoV Infecting Pregnant Women: Lessons from SARS, MERS, and Other Human Coronavirus Infections. Viruses. 2020; 12 (4): 194.
3. Zaigham M, Andersson O. Maternal and perinatal outcomes with COVID–19:A systematic review of 108 pregnancies. Acta Obstet Gynecol Scand. 2020; 99 (7): 823–9.
4. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet. 2020; 395 (10226): 809–15.
5. Sukhikh G, Petrova U, Prikhodko A, Starodubtseva N, Chingin K, Chen H, et al. Vertical Transmission of SARS-CoV-2 in Second Trimester Associated with Severe Neonatal Pathology. Viruses. 2021; 13 (3). DOI:10.3390/v13030447.
6. Ellington S, Strid P, Tong VT, Woodworth K, Galang RR, Zambrano LD, et al. Characteristics of women of reproductive age with laboratory-confirmed SARS-COV-2 infection by pregnancy status — United States, January 22-June 7, 2020. Morb Mortal Wkly Rep. 2020; 69 (25): 769–5.
7. Vivanti AJ, Vauloup-Fellous C, Prevot S, Zupan V, Suffee C, Do Cao J, et al. Transplacental transmission of SARS-CoV-2 infection. Nat Commun. 2020; 11 (1). DOI: 10.1038/s41467020-17436-6.
8. Shen B, Yi X, Sun Y, Bi X, Du J, Zhang C, et al. Proteomic and Metabolomic Characterization of COVID-19 Patient Sera. Cell. 2020; 182 (1): 59–72.
9. McGarrah RW, Crown SB, Zhang G, Shah SH, M.H.S., Newgard CB. Cardiovascular Metabolomics. Circ Res. 2018; 122 (9): 1238–58. DOI: 10.1161/CIRCRESAHA.117.311002.
10. Banoei MM, Vogel HJ, Weljie AM, Kumar A, Yende S, Angus DC, et al. Plasma metabolomics for the diagnosis and prognosis of H1N1 influenza pneumonia. 2017; 1–15.
11. Inoue S, Ikeda H. Differences in plasma amino acid levels in patients with and without bacterial infection during the early stage of acute exacerbation of COPD. Int J COPD. 2019; 14: 575–83.
12. Moat SJ, George RS, Carling RS. Use of Dried Blood Spot Specimens to Monitor Patients with Inherited Metabolic Disorders. Int J Neonatal Screen. 2020; 6 (2): 1–17.
13. Páez-Franco JC, Torres-Ruiz J, Sosa-Hernández VA, Cervantes-Díaz R, Romero-Ramírez S, Pérez-Fragoso A, et al. Metabolomics analysis reveals a modified amino acid metabolism that correlates with altered oxygen homeostasis in COVID-19 patients. Sci Rep. 2021; 11 (1). DOI: 10.1038/s41598-021-85788-0.
14. Rees CA, Rostad CA, Mantus G, Anderson EJ, Chahroudi A, Jaggi P. Altered amino acid profile in patients with SARS-CoV-2 infection. 2021; 118 (25): 4–6.
15. Hirschel J, Vogel M, Baber R, Garten A, Beuchel C, Dietz Y, et al. Relation of whole blood amino acid and acylcarnitine metabolome to age, sex, BMI, puberty, and metabolic markers in children and adolescents. Metabolites. 2020; 10 (4). DOI: 10.3390/metabo10040149.
16. Lomova NA, Chagovets VV, Dolgopolova EL, Novoselova AV, Petrova UL, Shmakov RG, et al. Changes in amino acid profile of cord blood plasma and amniotic fluid of mothers with COVID-19. Bulletin of Russian State Medical University. 2021; (3): 12–22. DOI: 10.24075/BRSMU.2021.032
17. Diorio C, McNerney KO, Lambert M, Paessler M, Anderson EM, Henrickson SE, et al. Evidence of thrombotic microangiopathy in children with SARS-CoV-2 across the spectrum of clinical

presentations. Blood Adv. 2020; 4 (23): 6051–63.
18. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nature Reviews Immunology. 2020; 20 (6): 363–74.
19. Gambardella J, Khondkar W, Morelli MB, Wang X, Santulli G, Trimarco V. Arginine and endothelial function. Biomedicines. 2020; 8 (8): 277.
20. Rodríguez PC, Ochoa AC. Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: Mechanisms and therapeutic perspectives. Immunological Reviews. 2008; 222 (1): 180–91.
21. Morris CR, Hamilton-Reeves J, Martindale RG, Sarav M, Ochoa Gautier JB. Acquired Amino Acid Deficiencies: A Focus on Arginine and Glutamine. In: Nutrition in Clinical Practice. SAGE Publications Inc., 2017; 30S–47S.
22. IKEDA H. Plasma amino acid levels in individuals with bacterial pneumonia and healthy controls. 2020; 1–17.
23. Ware LB, Magarik JA, Wickersham N, Cunningham G, Rice TW, Christman BW, et al. Low plasma citrulline levels are associated with acute respiratory distress syndrome in patients with severe sepsis. Crit Care. 2013; 17 (1): 1–8.
24. Morris SM Jr. Arginine: beyond protein. Am J Clin Nutr. 2006; 83: 508S–12S.
25. Schimke RT. Repression of enzymes of arginine biosynthesis in mammalian tissue culture. Biochim Biophys Acta. 1962; 62: 599–601.
26. Jackson MJ, Allen SJ, Beaudet AL, O'Brien WE. Metabolite regulation of argininosuccinate synthetase in cultured human cells. J Biol Chem. 1988; 263: 16388–94.
27. Lee J, Ryu H, Ferrante RJ, Morris SM Jr, Ratan RR. Translational control of inducible nitric oxide synthase expression by arginine can explain the arginine paradox. Proc Natl Acad Sci USA. 2003; 100: 4843–8.
28. Taheri F, Ochoa JB, Faghiri Z, Culotta K, Park HJ, Lan MS, et al. L-Arginine regulates the expression of the T-cell receptor zeta chain (CD3zeta) in Jurkat cells. Clin Cancer Res. 2001; 7: 958s–65s.
29. Fernandez J, Lopez AB, Wang C, Mishra R, Zhou L, Yaman I, et al. Transcriptional control of the arginine/lysine transporter, cat-1, by physiological stress. J Biol Chem. 2003; 278: 50000–9.
30. Cheng J, Wang F, Yu DF, Wu PF, Chen JG. The cytotoxic mechanism of malondialdehyde and protective effect of carnosine via protein cross-linking/mitochondrial dysfunction/reactive oxygen species/MAPK pathway in neurons. European Journal of Pharmacology. 2011; 650, 184–94.