GUT MICROBIOTA ALTERATIONS AND THEIR RELATIONSHIP TO THE DISEASE SEVERITY AND SOME CYTOKINE PROFILE INDICATORS IN PATIENTS WITH COVID-19

Gumenyuk LN, Golod MV, Silaeva NV, Sorokina LE ✉, Ilyasov SS, Androschyuk NA, Krivoshapko OR, Velilyaev AM, Asanova LN

Vernadsky Crimean Federal University, Simferopol, Russia

Gut microbiota is an essential element of maintaining the immune homeostasis, including in individuals with COVID-19. The study was aimed to assess taxonomic changes in the gut microbiota and their relationship with the disease severity and the levels of IL6, IL10, IL17, and TNFα in patients with COVID-19. A total of 110 patients with COVID-19 (index group) and 98 individuals with no COVID-19 (control group) were enrolled to the comparative cross-sectional study. The gut micribiota composition was determined by shotgun sequencing. Blood serum levels of IL6, IL10, IL17, and TNFα were assessed by enzyme-linked immunosorbent assay. The following significant changes in the gut microbiota composition were observed in patients with COVID-19 in contrast to controls: decreased abundance of *B. adolescentis* ($p = 0.048$), *E. rectale* ($p = 0.036$), *F. prausnitzi* ($p = 0.0002$), *B. dorei* ($p < 0.001$), and increased abundance of *R. gnavus* ($p = 0.012$), *Cl. hathewayi* ($p = 0.003$), *E. faecium* ($p = 0.0003$). Correlations were established between the abundance of *B. dorei* and the IL6 levels ($r = 0.49$; $p = 0.034$), the abundance of *F. prausnitzii* and the levels of IL10, IL17 ($r = 0.44$; $p = 0.001$ and $r = –0.52$; $p < 0.001$, respectively). The abundance of *R. gnavus* correlated with the TNFα levels, and the abundance of *E. faecium* was related to the levels of IL6 ($r = 0.47$; $p = 0.002$) and TNFα ($r = 0.56$; $p = 0.001$). The relationship between the abundance of *B. dorei*, *F. prausnitzii*, *E. faecium* and the higher SHOKS-COVID clinical assessment scale scores was also revealed ($r = –0.54$; $p = 0.001$, $r = –0.60$; $p < 0.001$ and $r = 0.67$; $p = 0.005$, respectively). Targeted correction of gut microbiota may improve the COVID-19 treatment efficacy.

Keywords: COVID-19, SARS-CoV-2, gut microbiota, cytokine status

Author contribution: Gumenyuk LN, Sorokina LE — significant contribution to the study concept and design; Golod MV, Silaeva NV, Androschyuk NA — data acquisition, analysis, and interpretation; Ilyasov SS — statistical data processing; Krivoshapko OR, Velilyaev AM, Asanova LN — manuscript writing.

Compliance with ethical standards: the study was approved by the Ethics Committee of the S.I. Georgievsky Medical Academy, V.I. Vernadsky Crimean Federal University (protocol № 11 dated November 23, 2021), planned and conducted in accordance with the Declaration of Helsinki. The informed consent was obtained from all study participants.

✉ **Correspondence should be addressed:** Leya E. Sorokina
Bulvar Lenina, 5/7, Simferopol, 295006, Republic of Crimea; leya.sorokina@mail.ru

Received: 18.01.2022 **Accepted:** 02.02.2022 **Published online:** 19.02.2022

DOI: 10.24075/brsmu.2022.006

ИЗМЕНЕНИЯ МИКРОБИОТЫ КИШЕЧНИКА И ИХ СВЯЗЬ С ТЯЖЕСТЬЮ ЗАБОЛЕВАНИЯ И НЕКОТОРЫМИ ПОКАЗАТЕЛЯМИ ЦИТОКИНОВОГО ПРОФИЛЯ У ПАЦИЕНТОВ С COVID-19

Л. Н. Гуменюк, М. В. Голод, Н. В. Силаева, Л. Е. Сорокина ✉, С. С. Ильясов, Н. А. Андрощук, О. Р. Кривошапко, А. М. Велиляев, Л. Н. Асанова

Крымский федеральный университет имени В. И. Вернадского, Симферополь, Россия

Важной составляющей поддержания иммунного гомеостаза в том числе при COVID-19 является микробиота кишечника. Целью работы было изучить изменения таксономического состава микробиоты кишечника и характер их взаимосвязи с тяжестью заболевания, содержанием IL6, IL10, IL17 и TNFα у пациентов с COVID-19. В одномоментном сравнительном исследовании приняли участие 110 пациентов с COVID-19 (основная группа) и 98 лиц, не инфицированных COVID-19 (контрольная группа). Оценку состава микробиоты кишечника проводили методом шотган-секвенирования. Уровень IL6, IL10, IL17 и TNFα в сыворотке крови определяли с помощью твердофазного иммуноферментного анализа. Обнаружены статистически значимые изменения в составе кишечной микробиоты у пациентов с COVID-19 в отличие от контрольной группы: снижение численности *B. adolescentis* ($p = 0,048$), *E. rectale* ($p = 0,036$), *F. prausnitzi* ($p = 0,0002$), *B. dorei* ($p < 0,001$) и повышение численности *R. gnavus* ($p = 0,012$), *Cl. hathewayi* ($p = 0,003$), *E. faecium* ($p = 0,0003$). Установлена корреляция численности *B. dorei* с показателем IL6 ($r = 0,49$; $p = 0,034$), численности *F. prausnitzii* и показателей IL10, IL17 ($r = 0,44$; $p = 0,001$ и $r = –0,52$; $p < 0,001$ соответственно). Численность *R. gnavus* коррелировала с показателем TNFα, а численность *E. faecium* — с IL6 ($r = 0,47$; $p = 0,002$) и TNFα ($r = 0,56$; $p = 0,001$). Также выявлена сопряженность численности *B. dorei*, *F. prausnitzii* и *E. faecium* с более высокими баллами по шкале оценки клинического состояния ШОКС-КОВИД ($r = –0,54$; $p = 0,001$, $r = –0,60$; $p < 0,001$ и $r = 0,67$; $p = 0,005$ соответственно). Направленная коррекция кишечной микробиоты может улучшить эффективность лечения COVID-19.

Ключевые слова: COVID-19, SARS-CoV-2, микробиота кишечника, цитокиновый статус

Вклад авторов: Л. Н. Гуменюк, Л. Е. Сорокина — существенный вклад в замысел и дизайн исследования; М. В. Голод, Н. В. Силаева, Н. А. Андрощук — сбор, анализ и интерпретация данных; С. С. Ильясов — статистическая обработка данных; О. Р. Кривошапко, А. М. Велиляев, Л. Н. Асанова — подготовка рукописи.

Соблюдение этических стандартов: исследование одобрено этическим комитетом Крымской медицинской академии им. С. И. Георгиевского» ФГАОУ ВО «Крымский федеральный университет им. В. И. Вернадского» (протокол № 11 от 23 ноября 2021 г.), спланировано и проведено в соответствии с Хельсинской декларацией. Все лица, включенные в исследование, подписали добровольное информированное согласие.

✉ **Для корреспонденции:** Лея Евгеньевна Сорокина
бул. Ленина, 5/7, г. Симферополь, 295006, Республика Крым; leya.sorokina@mail.ru

Статья получена: 18.01.2022 **Статья принята к печати:** 02.02.2022 **Опубликована онлайн:** 19.02.2022

DOI: 10.24075/vrgmu.2022.006

World Health Organization declared the pandemic in response to the novel coronavirus infection, caused by the SARS-CoV-2 virus, which was named COVID-19 [1]. Severe forms of this disorder are associated with progressive viral pneumonia and acute respiratory distress syndrome (ARDS). The increasing systemic inflammation plays a vital part in the COVID-19 pathophysiology. Excessive cytokine production, which is caused by SARS-CoV-2 and known as cytokine storm, is closely related to the disease severity [2]. High levels of interleukins IL1α, IL1RA, IL6, IL8, IL9, IL10, IL17, C-reactive protein (CRP), tumor necrosis factor (TNFα), are found in patients with COVID-19 [3–5].

Accumulated data of the *in vitro* and *in vivo* studies show that gastrointestinal tract (GIT) is also susceptible to COVID-19. For

example, studying the *in vitro* model, simulating the cellular and spatial intestinal structure, showed the SARS-CoV-2 capability of infecting enterocytes [6]. The frequency of gastrointestinal symptoms in patients with COVID-19 reaches 20% [7]. This is caused by the SARS-CoV-2 ability to enter cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor, which is intensively expressed on the enterocyte surface in the ileum and colon. The latter results in gastrointestinal symptoms due to the virus-induced immune-mediated damage [8]. Moreover, viral RNA of SARS-CoV-2 can be detected in fecal samples more than 30 days after the disease onset [9]. In this regard the role played by gut microbiota is actively discussed in literature. As is known, gut microbiota is an essential element of maintaining the immune homeostasis. For its part, gut microbiota dysbiosis is directly related to numerous inflammatory disorders [10]. Strong evidence of quantitative changes in the gut microbiota of patients with COVID-19 is provided [11]. In general, a downward trend in the bacterial species diversity is observed in patients with COVID-19, together with the depletion of beneficial commensals and enrichment in pathobionts [12]. However, information about the species composition of gut microbiota is fragmented and conflicting. Taking into account the potential of interactions, realized through the gut–lung axis, to increase the host susceptibility to viral infections and reduce the functional activity of immune cells, thus cotributing to systemic hyperinflammation and cytokine storm syndrome, further investigation of the gut microbiota alterations and their relatioship with the cytokine status indicators in patients with COVID-19 is relevant.

The study was aimed to assess taxonomic changes in the gut microbiota and their relationship with the disease severity and the levels of IL6, IL10, IL17, and TNFα in patients with COVID-19.

METHODS

A total of 110 patients with COVID-19 (66 females (60.0%), 44 males (40.0%); the average age was 28.6 ± 8.4 years), who had been admitted to hospitals, working in the Obligatory Medical Insurance system in Simferopol (index group, IG), and 98 healthy volunteers with no COVID-19 (61 females (62.2%), 37 males (37.8%); the average age was 29.2 ± 7.6 years), who had their annual health examination at the Gemokod medical center, Simferopol (control group, CG), were enrolled to the comparative cross-sectional study by continuous sampling.

Inclusion criteria for the IG: age 18–45 years; COVID-19, confirmed by positive polymerase chain reaction (PCR) test for the SARS-CoV-2 RNA and/or the typical multislice computed tomography (CT) findings of viral pneumonia; mild, moderate or severe COVID-19.

Exlusion criteria for the IG: extremely severe COVID-19; type 1 or 2 diabetes mellitus; obesity; myocardial infarction, severe cardiac arrhythmias, heart failure; history of hypertensive disease, stroke, transient ischemic attack; acute cerebrovascular disease (within six months before the beginning of the study); severe or decompensated concomitant somatic diseases, which could make it more difficult for the patient to participate in the study and affect the study results; irritable bowel syndrome; chronic gastrointestinal and liver diseases; hematological and oncological diseases; history of mental disorders, alcoholism or drug addiction; taking antibiotics, probiotics, prebiotics, antiviral drugs, symbiotics or acid–suppression medications within three months before the beginning of the study; taking medications, affecting the passage of stool, within a month before the beginning of the study; refusal to participate in research.

Inclusion criteria for healthy volunteers: age 18–45 years; no COVID-19, confirmed by PCR test for the SARS-CoV-2 RNA; no chronic disorders or allergic reactions; no infectious or acute disorders within two months before the study; no history of mental disorders, alcoholism or drug addiction; no abnormal passage of stool (constipation/diarrhea); taking no antibiotics, probiotics, prebiotics, antiviral drugs or symbiotics within three months before the beginning of the study; taking no medications, affecting the passage of stool, within a month before the beginning of the study.

Exlusion criteria for healthy volunteers: body temperature above 36.9 °C; refusal to participate in research.

Characteristics of patients with COVID-19 and healthy volunteers are presented in Table 1. The groups were comparable in gender (p = 0.96; χ^2), age (p = 0.92; χ^2), and body mass index (p = 0.054; χ^2).

Clinical characteristics of patients with COVID-19 are provided in Table. 2. Individuals with moderate COVID-19 (80 patients (72.7 ± 0.68%)) predominated among patients. Fever (103 patients (94.2%)) and cough (96 patients (86.9 ± 0.61%)) were the most common symptoms of the disease. According to CT images, viral pneumonia was diagnosed in 95 patients (67.1 ± 0.38%).

In all patients, the diagnosis of COVID-19 was confirmed by PCR test for SARS-CoV-2 (specimens were obtained from nasopharyngeal and oropharyngeal swabs) and/or typical multislice CT findings of viral pneumonia. The diagnosis and severity of COVID-19, as well as the extent of pneumonia on CT images, were assessed in accordance with the Temporary Guidelines on Prevention, Diagnosis and Treatment of Novel Coronavirus Infection (COVID-19) of the Ministry of Health of the Russian Federation, versions 6–9.

The original Clinical Assessment Scale for patients with coronavirus infection (SHOKS-COVID) was used to objectify the severity of clinical manifestations [13].

In order to analyze the taxonomic composition of the patients' gut microbiota, fecal samples were collected on the first day of hospital stay (in the morning, 8 a.m. to 10 a.m.). The samples were frozen and stored in disposable plastic containers at a temperature of –80 °C before the metagenomic analysis. Total DNA was isolated by the phenol-based extraction. Nucleotide sequence of the DNA isolated was determined by shotgun sequencing using the SOLiD 5500 Wildfire system for high-throughput sequencing (AppliedBiosystems; USA) [14].

QIIME software version 1.9.1 was used to filter the reads based on their quality and perform taxonomic classification [15]. The approach, involving the use of two taxonomic databases, was applied to perform the taxonomic assignment of the reads. At the first stage, the reference set of the bacterial

Table 1. Characteristics of patients with COVID-19 and healthy volunteers

Indicators	Index group (n = 110)	Control group (n = 98)
Females / males (n, %)	66 (60.0) / 44 (40.0)	61 (62.2) / 37 (37.8)
Average age years (M ± CD)	28.6 ± 8.4	29.2 ± 7.6
Body mass index, kg/m^2 (M ± CD)	23.4 ± 4.2	23.7 ± 3.6

Table 2. Clinical characteristics of patients with COVID-19

Indicator	Index group (n = 110)
Mild course (n, %)	7 (6.3)
Moderate course (n, %)	80 (72.7)
Severe course (n, %)	23 (21.0)
Fever	103 (94.2)
Sore throat	68 (61.8)
Cough	96 (86.9)
Shortness of breath	63 (56.9)
Nausea	37 (33.6)
Diarrhea	24 (22.1)
Temperature, median [25%; 75%]	37.8 [36.7; 37.9]
RR per minute, median [25%; 75%]	20.0 [17.0; 22.0]
HR, beats/min ($m \pm$ CD)	89.9 ± 16.1
SBP, mm Hg, median [25%; 75%]	120 [120; 132]
SO_2, %, median [25%; 75%]	96.0 [94.0; 98.0]
Severity of the condition, SHOKS-COVID score, points, Me [25%; 75%]	3.4 [1.6; 6.1]
CT stage — 0 (n, %)	25 (22.6)
CT stage CT–1 (n, %)	41 (37.4)
CT stage CT–2 (n, %)	32 (29.1)
CT stage CT–3 (n, %)	12 (10.9)

Note: RR — respiratory rate, HR — heart rate, SBP — systolic blood pressure, SO_2 — oxygen saturation, CT — computed tomography.

operational taxonomic units (OTUs) was selected based on the comparison of the 16s rRNA gene reads obtained with the GreenGenes database, version 13.5 [16]. At the second stage, taxonomic assignment of these OTUs was performed using the RDP algorithm based on the custom human intestinal database, HITdb [17].

Qualitative and quantitative asessment of the gut microbiota composition involved identification of species, genera, and phyla of microorganisms. Assessment of α-diversity by calculating the Chao1 index, number of the taxa observed (Sobs), and the indicator of species richness (ACE), was performed with the Mothur v.1.22.0 software (http:// www.mothur.org).

IL6, IL10, IL17 and TNFα were selected as the cytokine profile markers, which was due to their key role in the pathogenesis of cytokine storm and ARDS [3–5].

Blood samples were collected from peripheral vein during the first day of hospital stay. Blood was taken in the morning (between 7 and 9 a.m.) in a fasting state at rest (for at least 15 min). Test tubes containing blood serum were frozen and stored at a temperature of –20 °C. Serum levels of IL6, IL10, IL17, and TNFα were assessed using the enzyme-linked immunosorbent assay (ELISA) test system (Vector-Best; Vector-Best; Novosibirsk, Russia) with the Elisys Quattro automated ELISA analyzer (Human GmbH; Germany).

Statistical data processing was performed using the STATISTICA 8.0 software package (StatSoft. Inc.; USA). In case of normal distribution, the mean and the standard deviation were defined, and in case of non-normal distribution, the median, 25th and 75th percentiles were calculated. Distributions were tested for normality using the Gaussian distribution. Percentage and absolute values were defined for qualitative traits. Comparative analysis for the normal distribution of quantitative traits was performed using the parametric Student's t-test. In case of non-normal distribution it was performed using the Mann–Whitney U test, and comparative analysis of quantitative traits was carried out using the chi-squared test (χ^2). Spearman's correlation coefficient was used to assess the relationship between the traits. The differences were considered significant when $p < 0.05$. Correlation analysis and multiple rank correlation were also applied, and the correlations' significance was tested with the contingency tables.

RESULTS

Assessing the taxonomic composition of gut microbiota revealed a significant decrease in the α-diversity of the bacterial community (Chao1 index; $p = 0.016$) in patients with COVID-19 compared to controls. The ACE and Sobs indices were also slightly decreased in the group of patients with COVID-19 compared to the CG, however, no significant differences were observed ($p = 0.054$; $p = 0.052$, respectively) (Fig. 1).

Comparison of the species composition of gut mocrobiota revealed a significant decrease in the abundance of *Bifidobacterium adolescentis* SPM1005-A ($p = 0.048$), *Eubacterium rectale* ATCC 33656 ($p = 0.036$), *Faecalibacterium prausnitzi* A2-165 ($p = 0.0002$), *Bacterioides dorei* DSM 17855 ($p = 0.001$) in patients with COVID-19 compared to controls, together with the increased abundance of *Ruminococcus gnavus* ATCC 29149 ($p = 0.012$), *Clostridium hathewayi* DSM-13479 ($p = 0.003$), and *Enterococcus faecium* W54 ($p = 0.0003$) (Fig. 2).

Serum levels of IL6, IL10, IL17, and TNFα were significantly higher in patients with COVID-19 compared to the CG (Table 3).

Clarification of the relationship between the gut microbiota alterations and some cytokine profile indicators in patients with COVID-19 revealed significant correlations between the abundance of *B. dorei* and the IL6 levels ($r = –0.49$; $p = 0.034$), abundance of *F. prausnitzi* and the IL10, IL17 levels ($r = –0.44$; $p = 0.001$ and $r = –0.52$; $p < 0.001$, respectively). The abundance of *R. gnavus* correlated with the levels of TNFα ($r = 0.51$; $p = 0.036$), and the abundance of *E. faecium* was related to the levels of IL6 ($r = 0.47$; $p = 0.002$) and TNFα ($r = 0.56$; $p = 0.001$). Correlation analysis also revealed the relationship between the abundance of *B. dorei*, *F. prausnitzi*, *E. faecium*, and the higher SHOKS-COVID scores ($r = –0.54$ and $p = 0.001$; $r = –0.60$ and $p < 0.001$; $r = 0.67$ and $p = 0.005$, respectively).

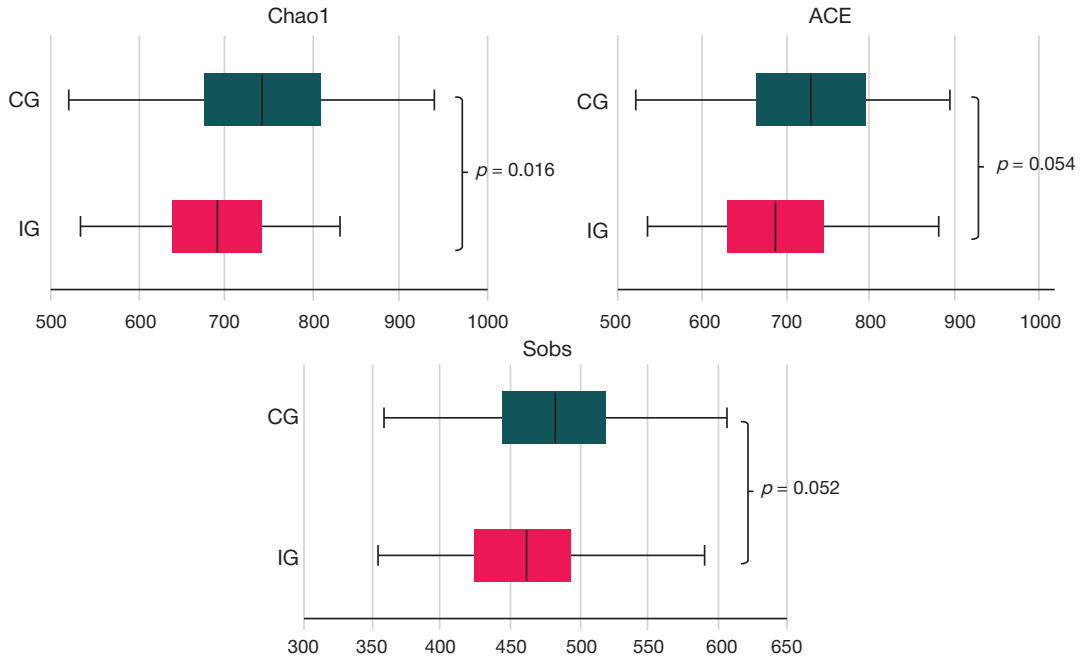

Fig. 1. Comparative analysis of the gut microbiota phylogenetic composition in patients with COVID-19 and healthy volunteers. IG — index group, CG — control group

DISCUSSION

In a number of previous studies, alterations in the composition of gut microbiota in patients with COVID-19 have been reported [7, 11, 12]. Our findings have also shown significant differences in the composition of gut microbiota between patients with COVID-19 and individuals with no COVID-19. According to our data, patients with COVID-19 have a lower bacterial α-diversity compared to individuals with no COVID-19, which is confirmed by the significantly lower Chao1 index and is consistent with the results of previous studies [11]. In patients with COVID-19, gut dysbiosis is characterized by the decreased abundance of bacteria having the immunomodulatory potential, the type representatives *B. adolescentis*, *E. rectale*, *F. prausnitzi*, *B. dorei*, that are known to be the main butyrate-producing bacteria (butyrate is a powerful anti-inflammatory metabolite). At the same time, we have revealed the increased abundance of potential pathobionts: the bacteria *R. gnavus*, *Cl. hathewayi*, and *E. faecium*. Among them, *E. faecium* is noteworthy, the presence of which in fecal samples of patients with COVID-19 has been previously reported by Italian researchers [18]. High levels of *E. faecium* in the gut microbiota of critically ill patients could be of some clinical significance due to the *E. faecium* pathogenic potential, resistance to common antimicrobial drugs, and the ability to rapidly acquire genetic material or alter gene expression, allowing the bacteria to acquire the

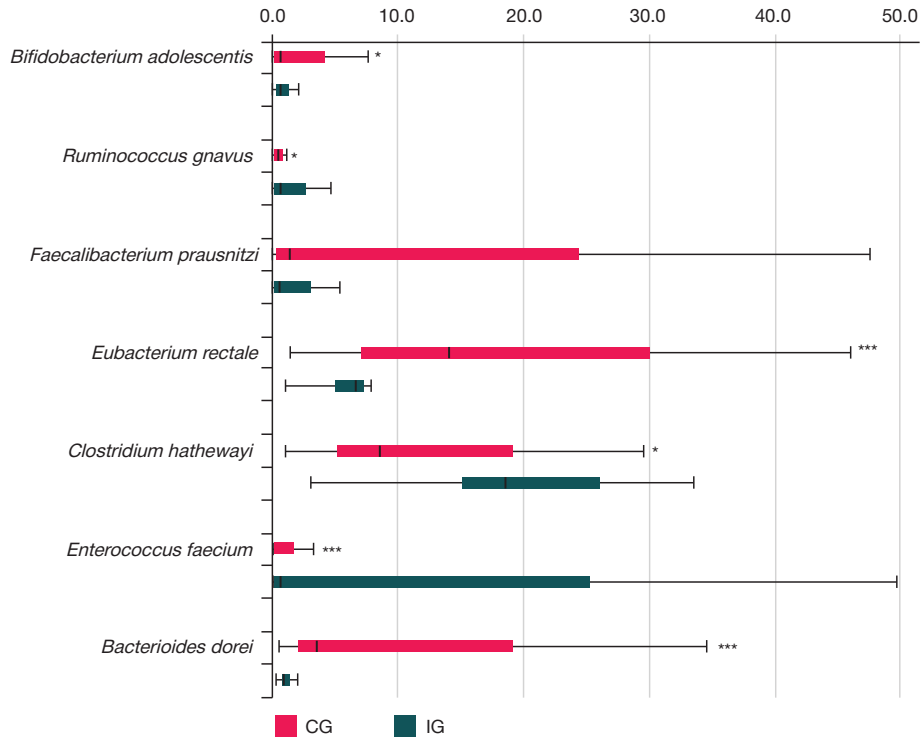

Fig. 2. Comparative analysis of the genus-level gut microbiota composition in patients with COVID-19 and healthy volunteers. IG — index group, CG — control group

Table 3. Comparative analysis of cytokine profile in patients with COVID-19 and healthy volunteers ($m \pm CD$)

Indicator	Index group $n = 110$	Control group $n = 98$	p
IL6, pg/mL	10.5 ± 6.6	3.8 ± 1.8	$p = 0.036$
IL10, pg/mL	9.3 ± 5.4	2.9 ± 1.1	$p = 0.021$
IL17, pg/mL	4.9 ± 2.2	2.4 ± 1.1	$p = 0.046$
TNFα, pg/mL	17.9 ± 8.6	5.2 ± 2.4	$p = 0.012$

resistance determinants to almost all antibacterial agents [19, 20]. Independently from the strain of the genus Enterococcus, gut microbiota of patients with COVID-19 can therefore function as a reservoir of the potentially antibiotic-resistant opportunistic pathogens, able to migrate through the damaged epithelial barrier into the systemic circulation, as has already been shown in the context of other disorders [20]. Our findings are to some extent consistent with the previously reported research data. For example, in one of the studies, patients with COVID-19 were characterized by the decreased abundance of *B. dorei*, and the increased abundance of *E. faecium* [18]. In another study, patients with COVID-19 were characterized by the decreased abundance of *B. adolescentis*, *E. rectale*, *F. prausnitzi*, and the increased abundance of bacteria *R. gnavus* [21]. The contrasting data patterns could be due to the fact, that the studies were carried out in different geographic regions with the use of different inclusion criteria. The groups varied considerably in age: 28.6 ± 8.4 years in our study vs. 73.0 [59.0; 85.0] in the previously reported study [18], and 36.4 ± 18.7 years in the study [21]. Moreover, in contrast to the listed above researhers, we did not study patients with somatic comorbidities and patients, taking antibacterial and/or antiviral medications, in order to mitigate their influence on the study results.

Despite the fact that some bacteria identified could be common to a number of other disorders, both gastrointestinal and systemic, the determined relationship between the decreased levels of *B. dorei*, *F. prausnitzi*, increased levels of *E. faecium* and the higher SHOKS-COVID scores suggests that changes in the abundance of these bacteria may be typical for this cohort of patients with COVID-19. We have compared the associations determined with the results of earlier studies. Thus, depletion of members of the genus Bacteroides was observed in patients, admitted to the intensive care unit [18]. It is interesting that in another study [7], the abundance of *B. dorei* negatively correlated with the SARS-CoV-2 load in the faeces of patients with COVID-19. The researchers noted that, taking into account the association of this bacterium with the decreased ACE2 receptor expression in the murine colon, *B. dorei* could potentially provide protection against the SARS-CoV-2 virus. Data are presented on the negative correlation between the bacterium *F. prausnitzii*, known for its anti-inflammatory effect, and the COVID-19 severity [7]. At the same time, higher abundance of strains of the genus *Enterococcus* ($p = 0.0001$) was observed in patients with COVID-19, admitted to the intensive care units, compared to patients, admitted to the general medicine units [18]. It can be assumed that terapeutic upscaling of *B. dorei*, *F. prausnitzii* together with the *E. faecium* downscaling is effective for mitigation of the disease severity. However, further research with appropriate design is required to confirm this hypothesis. Some authors point out that prebiotics and/or probiotics may be used as a potential (additional) vector of the COVID-19 therapy to reduce the disease severity and minimize the risk of secondary bacterial infections [22, 23].

A number of studies have proven the impact of gut microbiota on the susceptibility to infectious and noncommunicable diseases [24]. It has been specified that the intestinal flora immunomodulatory effect is realized through activation of genes, encoding a number of cytokines in the immune and epithelial cells, which determines heterogeneity of their immunomodulatory properties [25]. It has been reported that heterogeneity of the immunoregulatory effects is typical for both dominant and associative microsymbionts [26]. Thus, cumilative effects of the normal bacterial flora representatives on the secretion of cytokines by human immune cells in the conditions of eubiosis ensure the cytokine balance, characterized by moderate levels of pro-inflammatory cytokines (IL6, TNFα) and regulated by the suppressing effects of antiphlogogenic cytokines. Thus, in the context of increasing antigen load in dysbiosis through activation of toll-like receptors, the increased production of a whole range of pro-inflammatory cytokines is observed. These cytokines promote both local inflammation and the effector immune response in the gut-associated lymphoid tissue, protecting the body from pathogens [27].

As previously reported, COVID-19 infection is associated with the increased levels of cytokines IL6, IL10, IL17, and TNFα [4]. In our study, significant differences in the levels of IL6, IL10, IL17, and TNFβ between patients with COVID-19 and healthy volunteers were also observed.

It is assumed that the COVID-19 severity results from the cytokine storm [4]. It is noteworthy that some of the listed above cytokines correlate with the gut microbiota pattern, the specific profile of which is capable of inducing the cytokine storm. Our study revealed the correlation between the abundance of bacteria B. dorei and the levels of IL6 in patients with COVID-19.

We have revealed the relationship between the abundance of bacteria *F. prausnitzi* and the levels of IL10, which could be mediated by the decreased secretion of metabolites, blocking the NF-kB transcription factor activation and the IL8 production. For example, the in vivo study showed that activation of the peripheral blood mononuclear cells by *F. prausnitzi* resulted in the significantly reduced secretion of IL12 and TNFα, together with the increased secretion of IL10 [28]. Furthermore, the abundance of bacteria *F. prausnitzi* negatively correlated with the IL17 levels. When performing literature analysis, we have found no publications on studying the relationship between gut microbiota and IL17 in patients with COVID-19. At the same time, it has been shown that *F. prausnitzi* inhibits IL17 production in rats [29]. Studying the experimental colitis model has deminstrated that bacteria *F. prausnitzi* exert their anti-inflammatory effects due to production of butyrate, which maintains the balance between the pro-inflammatory T helper 17 cells (Th17) and immunoregulatory T cells (Treg) via inhibition of histone deacetylase 1. In turn, imbalance between Th17 and Treg promotes autoimmune inflammation [30].

We have also revealed the relationship between the abundance of the bacteria Ruminococcus gnavus and the TNFα levels. These data are consistent with the previously reported research results [31], showing that higher levels of *R. gnavus* in patients with COVID-19 correlate with the surge of pro-inflammatory cytokines IFNγ and TNFα, resulting in the cytokine storm and activation of the type 1 helper T cells.

We have discovered that the abundance of bacteria *E. faecium* correlates with the levels of IL6 and TNFα. We

have found the reports of similar correlations in patients with ulcerative colitis [32] and AIDS/HIV [33].

Thus, in the context of the COVID-19-associated gut dysbiosis, orientation of immunoregulatory effects towards pro- and anti-inflammatory cytokines is limited in the normal flora representatives, which contributes to the impaired homeostasis and the development of inflammatory and autoimmune responses.

CONCLUSIONS

The relationship between the decreased levels of bacteria *B. dorei*, *F. prausnitzi* together with the increased levels of bacteria *E. faecium* and the higher SHOKS-COVID scores has been defined in patients with COVID-19, which is indicative of the pathognomonic nature of these taxonomic alterations of intestinal microflora in individuals, infected with SARS-CoV-2. Significant correlations between the abundance of gut bacteria *B. dorei*, *F. prausnitzi*, *R. gnavus*, *E. faecium* and the levels of IL6, IL10, IL17, TNFα have been found in the SARS-CoV-2-infected patients, which confirms the gut microbiota capability of being involved in systemic inflammation and maintaining the immune tolerance in COVID-19. Our findings in combination with the data reported in literature demonstrate the key role in the COVID-19 pathogenesis played by microbiota. The more systematic and detailed research in this area would enable the development of new approaches, as well as selective probiotics for microbiota correction in patients with COVID-19 and treatment of post-COVID effects.

References

1. Statement on the second meeting of the International Health Regulations Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). World Health Organization (WHO), 2020. Available from: https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov) (assessed Dec 15, 2021).
2. Tao W, Zhang G, Wang X, et al. Analysis of the intestinal microbiota in COVID-19 patients and its correlation with the inflammatory factor IL18. Med Microecol. 2020; 5: 100023. DOI:10.1016/j.medmic.2020.100023.
3. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol. 2020; 20 (6): 363–74. DOI: 10.1038/s41577-020-0311-8.
4. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodríguez L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Cytokine Growth Factor Rev. 2020; 54: 62–75. DOI: 10.1016/j.cytogfr.2020.06.001.
5. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020; 395: 1054–62. DOI: 10.1016/S0140-6736(20)30566-3.
6. Lamers MM, Beumer J, van der Vaart J, et al. SARS-CoV-2 productively infects human gut enterocytes. Science. 2020; 369 (6499): 50–54. DOI: 10.1126/science.abc1669.
7. Zuo T, Zhang F, Lui GCY, et al. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. Gastroenterology. 2020; 159 (3): 944–55.e8. DOI:10.1053/j.gastro.2020.05.048.
8. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020; 382 (18): 1708–20. DOI: 10.1056/NEJMoa2002032.
9. Chan KH, Poon LL, Cheng VC, et al. Detection of SARS coronavirus in patients with suspected SARS. Emerg Infect Dis. 2004; 10 (2): 294–9. DOI: 10.3201/eid1002.030610.
10. Wu HJ, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes. 2012; 3 (1): 4–14. DOI: 10.4161/gmic.19320.
11. Gu S, Chen Y, Wu Z, et al. Alterations of the Gut Microbiota in Patients With Coronavirus Disease 2019 or H1N1 Influenza. Clin Infect Dis. 2020; 71 (10): 2669–78. DOI: 10.1093/cid/ciaa709.
12. Zuo T, Liu Q, Zhang F, et al. Depicting SARS-CoV-2 faecal viral activity in association with gut microbiota composition in patients with COVID-19. Gut. 2021; 70 (2): 276–84. DOI: 10.1136/gutjnl-2020-322294.
13. Mareev VYu, Begrambekova YuL, Mareev YuV. Kak ocenivat' rezul'taty lechenija bol'nyh s novoj koronavirusnoj infekciej (COVID-19)? Shkala Ocenki Klinicheskogo Sostojanija (ShOKS–KOVID). Kardiologija. 2020; 60 (11): 35–41. Russian.
14. Mitra S, Forster-Fromme K, Damms-Machado A, et al. Analysis of the intestinal microbiota using SOLiD16S rRNA gene sequencing and SOLiD shotgun sequencing. BMC Genomics. 2013; 14 (5): 16.
15. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7 (5): 335–6. DOI: 10.1038/ nmeth.f.303.
16. DeSantis, TZ, Hugenholtz P, Larsen N. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006; 72: 5069–72.
17. Ritari J, Salojärvi J, Lahti L, de Vos WM. Improved taxonomic assignment of human intestinal 16S rRNA sequences by a dedicated reference database. BMC Genomics. 2015; 16 (1): 1056. DOI: 10.1186/s12864-015-2265-y.
18. Gaibani P, D'Amico F, Bartoletti M, et al. The Gut Microbiota of Critically Ill Patients With COVID-19. Front Cell Infect Microbiol. 2021; 11: 670424. DOI: 10.3389/fcimb.2021.670424.
19. Gilmore MS, Clewell DB, Ike Y, Shankar N, eds. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Boston: Massachusetts Eye and Ear Infirmary, 2014.
20. Tamburini FB, Andermann TM, Tkachenko E, Senchyna F, Banaei N, Bhatt AS. Precision identification of diverse bloodstream pathogens in the gut microbiome. Nat Med. 2018; 24 (12): 1809–14. DOI: 10.1038/s41591-018-0202-8.
21. Yeoh YK, Zuo T, Lui GC, et al. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. Gut. 2021; 70 (4): 698–06. DOI: 10.1136/gutjnl-2020-323020.
22. Danilenko VN, Devyatkin AV, Marsova MV, et al Common in–ammatory mechanisms in COVID-19 and Parkinson's diseases: the role of microbiome, pharmabiotics and postbiotics in their prevention. Journal of Inflammation Research. 2021; 14, 6349–81. DOI: 10.2147/JIR.S333887.
23. Poluektova E, Yunes R, Danilenko V. The Putative Antidepressant Mechanisms of Probiotic Bacteria: Relevant Genes and Proteins. Nutrients. 2021; 13 (5): 1591. DOI: 10.3390/nu13051591.
24. West CE, Renz H, Jenmalm MC, et al. The gut microbiota and inflammatory noncommunicable diseases: associations and potentials for gut microbiota therapies. J Allergy Clin Immunol. 2015; 135 (1): 3–14. DOI: 10.1016/j.jaci.2014.11.012.
25. Sun Z, Song ZG, Liu C, et al. Gut microbiome alterations and gut barrier dysfunction are associated with host immune homeostasis in COVID-19 patients. BMC Med. 2022; 24: 20. DOI: 10.1186/s12916-021-02212-0.
26. Buharin OV, Chajnikova IN, Ivanova EV, i dr. Immunoreguljatornyj profil' mikrosimbiontov kishechnogo biotopa cheloveka. Zhurnal mikrobiologii, jepidemiologii i immunobiologii. 2018; 4: 42–51. Russian.
27. Hursitoglu M, Isıksacan N, Erismis B, et al. In-vitro cytokine production and nasopharyngeal microbiota composition in the early stage of COVID-19 infection. Cytokine. 2022; 149: 155757. DOI: 10.1016/j.cyto.2021.155757.

28. Sokol H, Pigneur B, Watterlot L, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci USA. 2008; 105 (43): 16731–6. DOI: 10.1073/pnas.0804812105.
29. Zhang M, Qiu X, Zhang H, et al. Faecalibacterium prausnitzii inhibits interleukin-17 to ameliorate colorectal colitis in rats. PLoS One. 2014; 9 (10): e109146. DOI: 10.1371/journal.pone.0109146.
30. Zhou L, Zhang M, Wang Y, et al. Faecalibacterium prausnitzii Produces Butyrate to Maintain Th17/Treg Balance and to Ameliorate Colorectal Colitis by Inhibiting Histone Deacetylase 1. Inflamm Bowel Dis. 2018; 24 (9): 1926–40. DOI: 10.1093/ibd/izy182.
31. van der Lelie D, Taghavi S. COVID-19 and the Gut Microbiome: More than a Gut Feeling. mSystems. 2020; 5 (4): e00453-20. DOI: 10.1128/mSystems.00453-20.
32. Li L, Zhong Q. Correlation of intestinal microflora with cytokines and Toll-like receptors expression in patients with ulcerative colitis. Infect Dis Inf. 2017; 30 (6): 361–4. DOI: 10.3969/j.issn.1007-8134.2017.06.012.
33. Lu J, Ma SS, Zhang WY, Duan JP. Changes in peripheral blood inflammatory factors (TNFα and IL6) and intestinal flora in AIDS and HIV-positive individuals. J Zhejiang Univ Sci B. 2019; 20 (10): 793–802. DOI: 10.1631/jzus.B1900075.

Литература

1. Statement on the second meeting of the International Health Regulations Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). World Health Organization (WHO), 2020. Available from: https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov) (assessed Dec 15, 2021).
2. Tao W, Zhang G, Wang X, et al. Analysis of the intestinal microbiota in COVID-19 patients and its correlation with the inflammatory factor IL18. Med Microecol. 2020; 5: 100023. DOI:10.1016/j.medmic.2020.100023.
3. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol. 2020; 20 (6): 363–74. DOI: 10.1038/s41577-020-0311-8.
4. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodríguez L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Cytokine Growth Factor Rev. 2020; 54: 62–75. DOI: 10.1016/j.cytogfr.2020.06.001.
5. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020; 395: 1054–62. DOI: 10.1016/S0140-6736(20)30566-3.
6. Lamers MM, Beumer J, van der Vaart J, et al. SARS-CoV-2 productively infects human gut enterocytes. Science. 2020; 369 (6499): 50–54. DOI: 10.1126/science.abc1669.
7. Zuo T, Zhang F, Lui GCY, et al. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. Gastroenterology. 2020; 159 (3): 944–55.e8. DOI:10.1053/j.gastro.2020.05.048.
8. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020; 382 (18): 1708–20. DOI: 10.1056/NEJMoa2002032.
9. Chan KH, Poon LL, Cheng VC, et al. Detection of SARS coronavirus in patients with suspected SARS. Emerg Infect Dis. 2004; 10 (2): 294–9. DOI: 10.3201/eid1002.030610.
10. Wu HJ, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes. 2012; 3 (1): 4–14. DOI: 10.4161/gmic.19320.
11. Gu S, Chen Y, Wu Z, et al. Alterations of the Gut Microbiota in Patients With Coronavirus Disease 2019 or H1N1 Influenza. Clin Infect Dis. 2020; 71 (10): 2669–78. DOI: 10.1093/cid/ciaa709.
12. Zuo T, Liu Q, Zhang F, et al. Depicting SARS-CoV-2 faecal viral activity in association with gut microbiota composition in patients with COVID-19. Gut. 2021; 70 (2): 276–84. DOI: 10.1136/gutjnl-2020-322294.
13. Мареев В. Ю., Беграмбекова Ю. Л., Мареев Ю. В. Как оценивать результаты лечения больных с новой коронавирусной инфекцией (COVID-19)? Шкала Оценки Клинического Состояния (ШОКС–КОВИД). Кардиология. 2020; 60 (11): 35–41.
14. Mitra S, Forster-Fromme K, Damms-Machado A, et al. Analysis of the intestinal microbiota using SOLiD16S rRNA gene sequencing and SOLiD shotgun sequencing. BMC Genomics. 2013; 14 (5): 16.
15. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7 (5): 335–6. DOI: 10.1038/ nmeth.f.303.
16. DeSantis, TZ, Hugenholtz P, Larsen N. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006; 72: 5069–72.
17. Ritari J, Salojärvi J, Lahti L, de Vos WM. Improved taxonomic assignment of human intestinal 16S rRNA sequences by a dedicated reference database. BMC Genomics. 2015; 16 (1): 1056. DOI: 10.1186/s12864-015-2265-y.
18. Gaibani P, D'Amico F, Bartoletti M, et al. The Gut Microbiota of Critically Ill Patients With COVID-19. Front Cell Infect Microbiol. 2021; 11: 670424. DOI: 10.3389/fcimb.2021.670424.
19. Gilmore MS, Clewell DB, Ike Y, Shankar N, eds. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Boston: Massachusetts Eye and Ear Infirmary, 2014.
20. Tamburini FB, Andermann TM, Tkachenko E, Senchyna F, Banaei N, Bhatt AS. Precision identification of diverse bloodstream pathogens in the gut microbiome. Nat Med. 2018; 24 (12): 1809–14. DOI: 10.1038/s41591-018-0202-8.
21. Yeoh YK, Zuo T, Lui GC, et al. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. Gut. 2021; 70 (4): 698–06. DOI: 10.1136/gutjnl-2020-323020.
22. Danilenko VN, Devyatkin AV, Marsova MV, et al Common in–ammatory mechanisms in COVID-19 and Parkinson's diseases: the role of microbiome, pharmabiotics and postbiotics in their prevention. Journal of Inflammation Research. 2021; 14, 6349–81. DOI: 10.2147/JIR.S333887.
23. Poluektova E, Yunes R, Danilenko V. The Putative Antidepressant Mechanisms of Probiotic Bacteria: Relevant Genes and Proteins. Nutrients. 2021; 13 (5): 1591. DOI: 10.3390/nu13051591.
24. West CE, Renz H, Jenmalm MC, et al. The gut microbiota and inflammatory noncommunicable diseases: associations and potentials for gut microbiota therapies. J Allergy Clin Immunol. 2015; 135 (1): 3–14. DOI: 10.1016/j.jaci.2014.11.012.
25. Sun Z, Song ZG, Liu C, et al. Gut microbiome alterations and gut barrier dysfunction are associated with host immune homeostasis in COVID-19 patients. BMC Med. 2022; 24: 20. DOI: 10.1186/s12916-021-02212-0.
26. Бухарин О. В., Чайникова И. Н., Иванова Е. В. и др. Иммунорегуляторный профиль микросимбионтов кишечного биотопа человека. Журнал микробиологии, эпидемиологии и иммунобиологии. 2018; 4: 42–51.
27. Hursitoglu M, Isıksacan N, Erismis B, et al. In-vitro cytokine production and nasopharyngeal microbiota composition in the early stage of COVID-19 infection. Cytokine. 2022; 149: 155757. DOI: 10.1016/j.cyto.2021.155757.
28. Sokol H, Pigneur B, Watterlot L, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci USA. 2008; 105 (43): 16731–6. DOI: 10.1073/pnas.0804812105.
29. Zhang M, Qiu X, Zhang H, et al. Faecalibacterium prausnitzii inhibits interleukin-17 to ameliorate colorectal colitis in rats. PLoS One. 2014; 9 (10): e109146. DOI: 10.1371/journal.pone.0109146.
30. Zhou L, Zhang M, Wang Y, et al. Faecalibacterium prausnitzii Produces Butyrate to Maintain Th17/Treg Balance and to Ameliorate Colorectal Colitis by Inhibiting Histone Deacetylase 1. Inflamm Bowel Dis. 2018; 24 (9): 1926–40. DOI: 10.1093/ibd/

izy182.
31. van der Lelie D, Taghavi S. COVID-19 and the Gut Microbiome: More than a Gut Feeling. mSystems. 2020; 5 (4): e00453-20. DOI: 10.1128/mSystems.00453-20.
32. Li L, Zhong Q. Correlation of intestinal microflora with cytokines and Toll-like receptors expression in patients with ulcerative colitis. Infect Dis Inf. 2017; 30 (6): 361–4. DOI: 10.3969/j.issn.1007-8134.2017.06.012.
33. Lu J, Ma SS, Zhang WY, Duan JP. Changes in peripheral blood inflammatory factors (TNFα and IL6) and intestinal flora in AIDS and HIV-positive individuals. J Zhejiang Univ Sci B. 2019; 20 (10): 793–802. DOI: 10.1631/jzus.B1900075.