УДК 616.361-006.6:616.34-093/-098
https://doi.org/10.20538/1682-0363-2025-1-105-113

Study of gut microbiota in cholangiocarcinoma patients

Fedorova O.S., Kovshirina A.E., Sokolova T.S., Kulenich V.V., Ogorodova L.M.

Siberian State Medical University
2, Moscow Trakt, Tomsk, 634050, Russian Federation

ABSTRACT

Aim. To analyze the taxonomic composition of the intestinal microbiota in patients with cholangiocarcinoma (CCA) and compare it to individuals without oncopathology.

Materials and methods. The study included patients with histologically verified cholangiocarcinoma (n = 30) and a control group (n = 27). An integrated approach was used, including clinical and anamnestic, laboratory, and instrumental methods. The intestinal microbiota was studied through amplicon sequencing of the bacterial 16S rRNA gene.

Results. The assessment of alpha- and beta-diversity of the microbiota in patients with CCA did not show any significant differences compared to the control group. However, a comparative analysis revealed changes in the representation of a number of microorganisms at different taxonomic levels, including a higher content of *Bacteroides* and *Lachnospiraceae_NK4A136_group* in patients with CCA. Additionally, bacteria that influence the change in the global balance of microorganisms were identified in both groups, such as *[Ruminococcus]_torques_group, Subdoligranulum, Parasutterella, unclassified Firmicutes* in samples of patients with CCA and *Oscillospiraceae* and *Erysipelotrichaceae UCG-006* in the control group.

Conclusion. The study found a number of significant differences in bacterial representation between patients with cholangiocarcinoma and control group participants. Further research on the intestinal microbiota has the potential to develop non-invasive tools for early diagnosis of CCA.

Keywords: cholangiocarcinoma, gut microbiota, amplicon sequencing of bacterial 16S rRNA, liver cancer

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The study was funded by the Russian Science Foundation (Agreement No. 23-25-00432).

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 9389 of 27.02.2023).

For citation: Fedorova O.S., Kovshirina A.E., Sokolova T.S., Kulenich V.V., Ogorodova L.M. Study of gut microbiota in cholangiocarcinoma patients. *Bulletin of Siberian Medicine.* 2025;24(1):105–113. https://doi.org/10.20538/1682-0363-2025-1-105-113.

Исследование микробиоты кишечника у больных холангиокарциномой

Федорова О.С., Ковширина А.Е., Соколова Т.С., Куленич В.В., Огородова Л.М.

Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2

РЕЗЮМЕ

Цель исследования: проанализировать таксономический состав кишечной микробиоты у пациентов с холангиокарциномой (ХК) по сравнению с индивидуумами без онкопатологии.

✉ *Fedorova Olga S.,* olga.se rgeevna.fedorova@gmail.com

Материалы и методы. В исследование включены пациенты с гистологически верифицированной холангиокарциномой (n = 30) и контрольная группа (n = 27). Для решения задач данного проекта использован комплексный подход, включающий клинико-анамнестические, лабораторные и инструментальные методы. Исследование микробиоты кишечника выполнено методом ампликонного секвенирования гена бактериальной 16S рРНК.

Результаты. При оценке альфа- и бета-разнообразия микробиоты у пациентов с ХК в сравнении с контрольной группой значимых различий не выявлено. Сравнительный анализ показал изменения в представленности ряда микроорганизмов на разных таксономических уровнях, в том числе более высокое содержание *Bacteroides* и *Lachnospiraceae_NK4A136_group* у пациентов с ХК. Также определены бактерии, оказывающие влияние на изменение глобального баланса микроорганизмов в образцах для пациентов с ХК *([Ruminococcus]_torques_group, Subdoligranulum, Parasutterella,* неклассифицированные *Firmicutes*), и контроля (*Oscillospiraceae и Erysipelotrichaceae UCG-006*).

З**аключение.** В результате исследования выявлен ряд значимых различий в представленности бактерий у пациентов с холангиокарциномой в сравнении с участниками контрольной группы. Дальнейшие исследования кишечной микробиоты представляют перспективу для разработки неинвазивных инструментов ранней диагностики ХК.

Ключевые слова: холангиокарцинома, кишечная микробиота, ампликонное секвенирование бактериальной 16S рРНК, рак печени

Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Источник финансирования. Исследование выполнено при поддержке Российского научного фонда, грант «Изменение микробиоты кишечника как фактор прогрессирования перидуктального фиброза и развития холангиокарциномы» (№ 23-25-00432).

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом СибГМУ (протокол № 9389 от 27.02.2023).

Для цитирования: Федорова О.С., Ковширина А.Е., Соколова Т.С., Куленич В.В., Огородова Л.М. Исследование микробиоты кишечника у больных холангиокарциномой. *Бюллетень сибирской медицины.* 2025;24(1):105–113. https://doi.org/10.20538/1682-0363-2025-1-105–113.

INTRODUCTION

According to world statistics, liver cancer holds the sixth position among the most common localizations of malignant neoplasms. The incidence of hepatobiliary cancer is increasing worldwide, accounting for 15% of all primary liver cancers and 3% of malignant neoplasms of the digestive tract [1]. Cholangiocellular carcinoma (cholangiocarcinoma, CCA) is one of the leading causes of mortality in oncology due to its aggressive nature, lack of specific symptoms, prolonged asymptomatic course and methods of preclinical diagnosis, and resistance to therapy [2].

The highest incidence of CCA in the world is registered in the countries of Southeast Asia and North Africa, with more than 20 cases per 100 thousand people annually [2]. In the Russian Federation, the highest incidence of CCA is observed in the regions of Western Siberia, where it is more than twice the world average (more than 9 cases per 100 thousand people annually) and poses a significant socioeconomic burden [2, 3].

CCA belongs to multifactorial diseases, in the development of which genetic, infectious, environmental, and epidemiological risk factors are involved. The most significant risk factors include primary sclerosing cholangitis/ulcerative colitis, chronic viral hepatitis C and B, hepatic trematodoses, Epstein-Barr virus carrier, non-alcoholic fatty liver disease, cholelithiasis, and (or) malformations of the biliary system, food carcinogens (N-dinitrosodimethylamine), deposits of X-ray contrast agents (thorotrast) in bile ducts [4–6]. Immune mechanisms play a crucial role in carcinogenesis: chronic inflammation leads to increased exposure of cholangiocytes to proinflammatory mediators – interleukin-6, tumor necrosis factor, cyclooxygenase-2 and activation of Wnt signaling pathway with progression of mutations

in tumor suppressor genes, proto-oncogenes and DNA repair genes, and increased risk of carcinogenesis [7].

According to clinical and experimental studies, the intestinal microbiota is the most important factor in the development of liver diseases along the gut – liver axis [8, 9]. Inflammatory reactions resulting from changes in the gut microbiome are associated with the development of a number of chronic non-communicable diseases and are also considered as a potential carcinogenic mechanism [10–13]. However, the fundamental mechanisms underlying this relationship are still unclear. Thus, it is essential to study the taxonomic composition of the intestinal microbiota in patients with CCA and individuals without cancer.

MATERIALS AND METHODS

To solve the set tasks, the study was conducted in a case-control design in parallel groups. The study protocol was approved by the Local Ethics Committee of Siberian State Medical University (Protocol No. 9389 of 27.02.2023).

The study included the following groups: patients with histologically confirmed CCA (n = 30) and participants without cancer and/or clinically significant changes in the hepatobiliary system (control group, n = 27). Among the patients with CCA, 16.6% received chemotherapy, 13.3% received a short course of antibiotic therapy (up to 7 days), and 33.3% underwent surgical intervention on the organs of the hepatobiliary system within 2-4 weeks prior to inclusion in the study. The control group did not receive any of these treatments during the specified periods.

CCA was diagnosed through the histologic and/or immunohistochemical examination of biopsy or postoperative liver material, according to the following ICD-10 codes: C22.1 – cancer of the intrahepatic bile duct; C24 – malignant neoplasm of other and unspecified parts of the biliary tract (n = 29); C24.0 – malignant neoplasm of the extrahepatic bile duct (n = 1). The inclusion criterion for all participants was a signed informed consent to participate in the study.

During the study, the medical histories and physical examination data of the participants were analyzed, including vital signs, anthropometric data, and system and organ examination data.

All participants underwent ultrasound examination of the hepatobiliary system using a high-resolution mobile ultrasound scanner (Mindray M7, Shenzhen Mindray Bio-Medical Electronics Co, Ltd, PRC) in accordance with the protocol proposed within the framework of epidemiologic studies of opisthorchiasis previously conducted in Southeast Asia [14]. We evaluated the liver size and structural features of the liver parenchyma, including echo signs of CCA and periductal fibrosis (defined as an increase in periportal echogenicity of more than 3 mm around the intrahepatic bile ducts of the second order), as well as dilation and thickening of the bile duct walls. The presence of liver masses was verified by computed tomography (CT) and/or magnetic resonance imaging (MRI) in patients with CCA.

To assess the composition of the intestinal microbiota, stool samples were collected in special sterile containers and stored at minus 80 degrees Celsius until analysis. Additionally, stool samples were evaluated for the presence of O. felineus infection through microscopy of two stool samples using Parasep concentrators (DiaSysLtd, UK).

To isolate DNA from the stool samples, we used the Nobias DNA Extraction Kit (Nobias Technologies LLC, Russia) with the extraction protocol, including the stage of stool sample homogenization with the help of solid particles (bead beating) and precipitation of inhibitors.

The microbiota of stool samples from patients with CCA and the control group was studied through amplicon sequencing of the V3-V4 fragment of the 16S rRNA gene. As a result of sample preparation and sequencing, 6 samples were excluded due to inadequate quality for analysis, and data from 51 patients' samples were included in the microbiota study data analysis. Sequencing of the V4 region of the bacterial 16S rRNA gene was performed on an Illumina MiSeq instrument.

Bioinformatic analysis of the obtained reads was performed using Qlime 2 software. Data were aggregated into ASVs (amplicon sequence variants) using the DADA2 plugin, and taxonomic analysis was performed using the RDP classifier plugin and the Silva taxonomy database. Statistical analysis was performed using R 4.3.3. Alpha diversity was assessed using the total number of operational taxonomic units (OTUs), Shannon, Chao1, and Simpson diversity indices (rbiom 1.0.3). Alpha diversity between groups was compared using the non-parametric Mann – Whitney test. Beta diversity was analyzed through nonparametric permutation analysis of variance

(PERMANOVA) using distance matrices based on the Eitchison distance and Jaccard's measure. Beta diversity differences were analyzed using the adonis test (vegan 2.6-4). Microbial representation between groups was compared using the nonparametric Mann – Whitney test and compositional analysis through the balance selection method (selbal 0.1.0) for two groups, and the Kraskell – Wallis criterion followed by Dunn's test (dunn.test 1.3.6) for three or more groups. Correlation analysis was performed using Spearman rank correlation (Hmisc 5.1-2). Benjamini – Hochberg multiple comparisons correction was applied to all statistical tests. The level of statistical significance was chosen at 0.05.

RESULTS

During the study, two clinical groups of patients were formed: patients with histologically verified CCA (n = 30) and a control group (n = 27). In the CCA group, 96.7% of cases were diagnosed with intrahepatic localization of cholangiocarcinoma, and only 1 patient had an extrahepatic Klatskin tumor; 46.7% of patients were diagnosed at stage I-II and 53.3% – at stage III-IV according to the TNM classification. Chemotherapy was given to 16.7% of patients and surgical treatment – to 30% of participants, while 53.3% received only palliative care.

The most frequent symptoms in patients with CCA were abdominal pain, bloating, positive bladder symptoms of Ker and Ortner, liver pain on palpation, as well as significantly higher levels of bilirubin, aspartate aminotransferase, alanine aminotransferase and detection of hepatomegaly, periductal fibrosis, and bile duct dilatation on ultrasound.

Neoplasms were detected by ultrasound and confirmed by abdominal MRI / CT.. All clinical data for the studied groups are presented in Table 1.

Table 1.

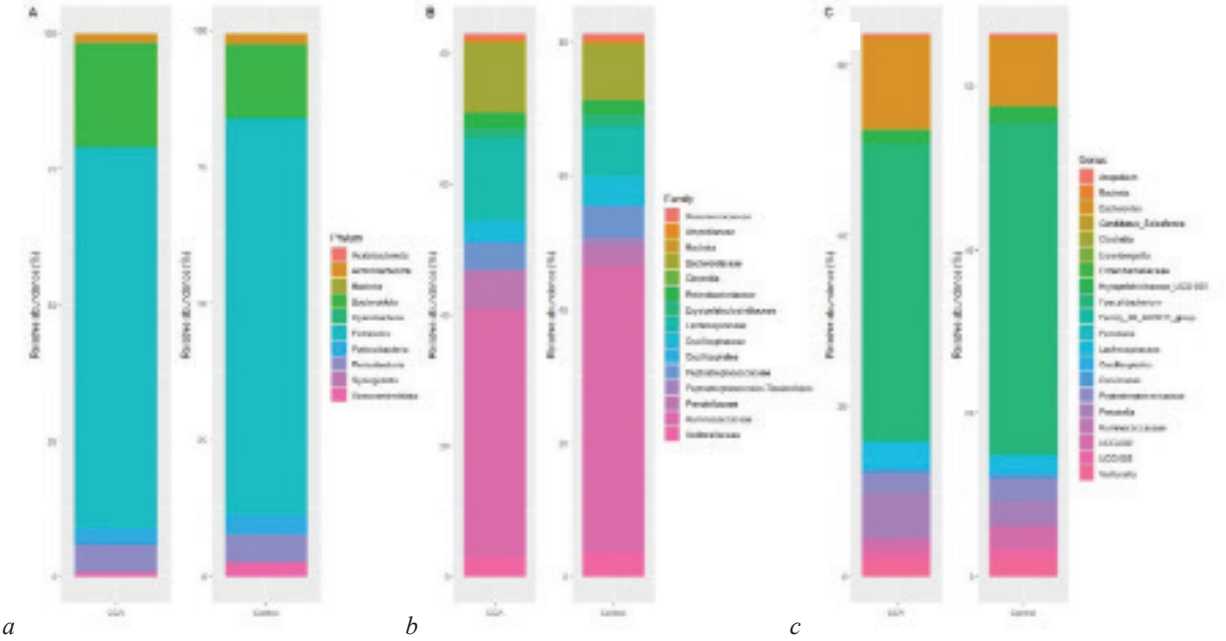
Clinical characteristics of the studied groups

Parameter	Patients with CCA (n = 30)	Control group (n = 27)	p
Demographic and anamnestic characteristics			
Gender, n (%) male female	 18 (60.0) 12 (40.0)	 14 (51.8) 13 (48.2)	$p > 0.05$
Age, years (N (Q_1; Q_2))	59.96 (54.0 ; 67.0)	62,8 (58.0 ; 68.0)	$p > 0.05$
Smoking, n (%)	21 (70.0)	11 (36.7)	$p < 0.05$
Average history of smoking (years)	12.2 (0.0 ; 20.0)	10.8 (0.0 ; 20.0)	$p > 0.05$
Alcohol consumption, n (%)	15 (50,0)	11 (36.7)	$p > 0.05$
Gastric and/or duodenal ulcer disease, n (%)	8 (26.7)	4 (14.8)	$p < 0.05$
Hypertension, n (%)	15 (50.0)	19 (70.4)	$p > 0.05$
Chronic heart failure, n (%)	4 (13.3)	9 (33.3)	$p < 0.05$
Type 2 diabetes mellitus, n (%)	3 (10.0)	4 (14.8)	$p > 0.05$
Cholelithiasis, n (%)	8 (26.7)	2 (7.4)	$p < 0.05$
Clinical characteristics			
Body mass index (kg/cm2)	26.6 (21.0 ; 28.4)	29.1 (23.0 ; 33.5)	$p > 0.05$
Fever, n (%)	4 (6.7)	–	
Jaundice of the skin and visible mucosae, n (%)	11 (36.7)	–	
Hepatomegaly on the liver palpation, n (%)	14 (43.3)	4 (15.4)	$p < 0.05$
Positive gall bladder symptoms, n (%)	10 (33.3)	–	
Biochemical markers			
Total protein, g/l	67.2 (62.0 ; 73.0)	68.7 (63.0 ; 75.0)	$p > 0.05$
Total bilirubin, mcmol/l	54.2 (8.9 ; 41.0)	11.7 (6.0 ; 14.2)	$p < 0.05$
Conjugated bilirubin, mcmol/l	28.4 (2.3 ; 32.0)	1.8 (0.0 ; 2.3)	$p < 0.05$
ALT, IU/I	61.8 (25.0 ; 73.0)	30.2 (14.0 ; 33.0)	$p < 0.05$
AST, IU/I	58.2 (32.0 ; 73.0)	30.5 (17.0 ; 42.0)	$p < 0.05$
Ultrasound examination of the hepatobiliary system			
Hepatomegaly, n (%)	8 (26.7)	2 (7.4)	$p < 0.05$
Dilation of the bile ducts, n (%)	14 (46.7)	–	
Thickening, irregular walls of bile ducts, n (%)	7 (23.3)	1 (3.7)	$p < 0.05$
Periductal fibrosis, n (%)	12 (40.0)	–	

BIOINFORMATICS AND STATISTICAL ANALYSIS OF GUT MICROBIOTA COMPOSITION DATA

The most represented types in the microbiota of stool samples from patients with CCA were *Firmicutes* (70.1%), *Bacteroidota* (18.9%), *Proteobacteria* (5.1%), *Patescibacteria* (2.9%), and *Actinobacteriota* (1.6%). The control group had a higher proportion of *Firmicutes* (72.6%), *Bacteroidota* (13.4%), *Proteobacteria* (4.7%), *Patescibacteria* (3.6%), and *Verrucomicrobiota* (2.5%, Fig. 1, *a*). At the family level, patients with CCA were dominated by *Ruminococcaceae* (38.0%), *Lachnospiraceae* (12.5%), *Bacteroidaceae* (10.7%), *Prevotellaceae* (5.9%), and *Peptostreptococcaceae* (4.1%). In the control group, the most represented families of microorganisms were the following: *Ruminococcaceae* (42.9%), *Bacteroidaceae* (8.3%), *Lachnospiraceae* (7.3%), *Peptostreptococcaceae* (4.7%), and *Oscillospiraceae* (4.5%, Fig. 1, *b*).

At the genus level, patients with CCA had a predominant rate of *Faecalibacterium* (34.7%), *Bacteroides* (10.7%), *Prevotella* (5.2%), unclassified genera of the families *Lachnospiraceae* (3.1%) and *Peptostreptococcaceae* (2.3%). In the control group, the most represented genera of microorganisms were *Faecalibacterium* (40.5%), *Bacteroides* (8.3%), *Prevotella* (3.0%), UCG-002 (2.7%) and unclassified genera of the family *Peptostreptococcaceae* (2.7%, Fig. 1, *c*).



a *b* *c*

Fig. 1. The bar graph shows the most represented microbial taxa in stool samples from patients with cholangiocarcinoma (CCA) and control groups (Control): *a* – at the order level, *b* – at the family level, *c* – at the genus level

The comparative analysis of alpha diversity of the microbiota of stool samples showed no statistically significant differences between the group of patients with CCA and the control group. (Fig. 2). Similarly, no differences were found between the groups when beta diversity was assessed.

The Mann – Whitney test was used to identify bacteria (28 taxa) whose abundance was statistically significantly different in the group of patients with CCA compared to the control group. The results showed that CCA is associated with an increase in the abundance of bacteria from the following orders: *Lachnospirales*, *Clostridiales*, *Rhodospirillales*, семейств *Lachnospiraceae*, *Tannerellaceae*, *Clostridiaceae*, unclassified *Rhodospirillales,* and *Bacteroidales,* compared to the control group. The intestinal microbiota of the control group participants was characterized by a higher content of bacteria from the *Staphylococcaceae* family and the genera of *Staphylococcus*, and *Finegoldia*. At the genus level, the microbiota samples of CCA patients were characterized by a higher content of *[Ruminococcus] torques group, Dorea, unclassified Lachnospiraceae, CAG-56, Agathobacter, Clostridium sensu stricto*

1, uncultured Rhodospirillales, Subdoligranulum, CAG-352, Anaerostipes, Parabacteroides, uncultured Bacteroidales, Anaerovoracaceae Family XIII AD3011 group, Oscillospiraceae NK4A214 group, and *Lachnospiraceae NK4A136 group.*

We also used the method of selecting balances with sex as a covariate to reduce the detection of false positives. Only bacteria that were in global balance (R^2 = 0.726, p = 0.006, Fig. 3) were considered significant.

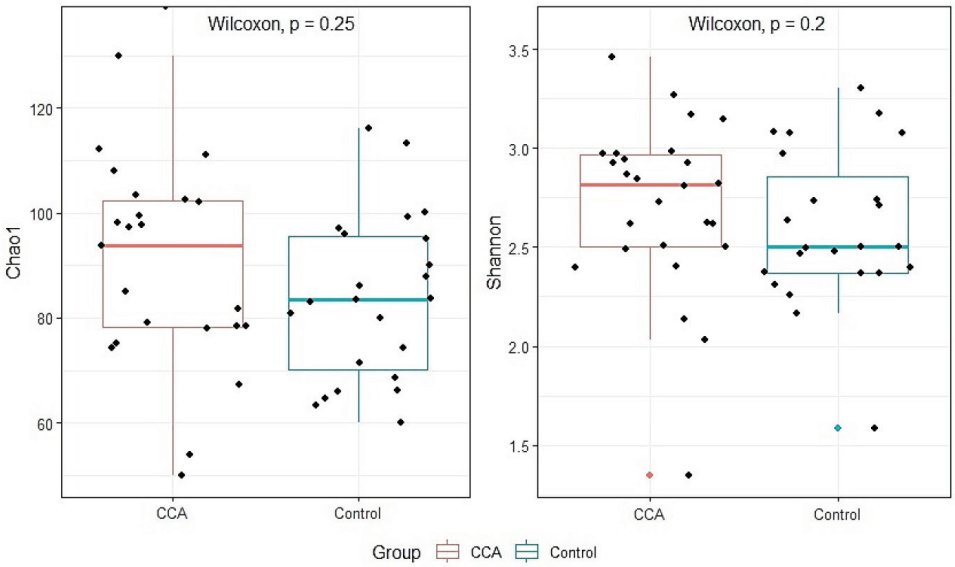


Fig. 2. The graph shows comparison of alpha diversity between cholangiocarcinoma (CCA) and the control group (Control) of patients in terms of number of operative taxonomic units (OTUs), Shannon, Chao1 and Simpson diversity indices. The *p*-value was calculated using the nonparametric Mann – Whitney test

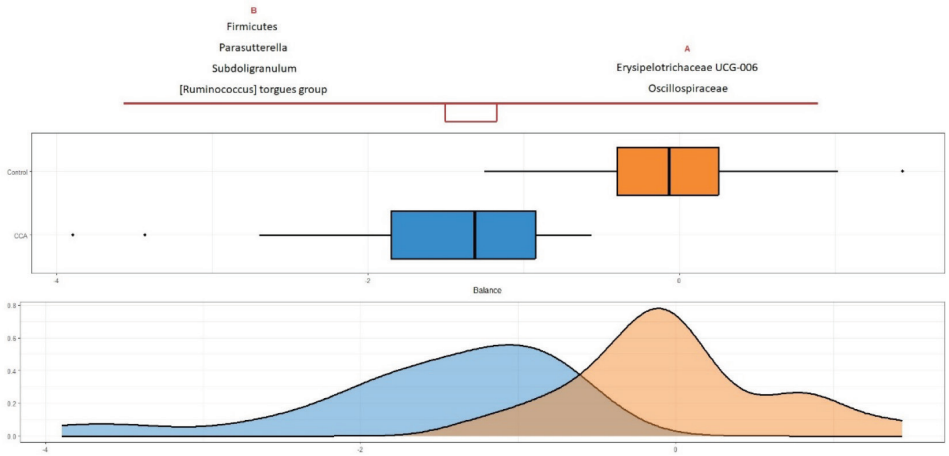


Fig. 3. The selection plot of microbial balances in samples at the genus level. The bacteria presented in the upper part of the graph influence the variation of the global balance of microorganisms in the samples. The middle and bottom parts show box-and-whisker type plots and density distribution curves of the balance for the groups of CCA patients (blue) and the control groups (orange)

Additionally, we performed a correlation analysis between bacteria in the global balance, taking into account the joint influence of several bacterial genera. We found a statistically significant mean correlation between bacteria from the genera *[Ruminococcus] torques group* and *Subdoligranulum* (r = 0.56, $p < 0.0001$), which changed the balance of microorganisms downward.

Within the group of patients with CCA, we investigated the differences in the relative bacterial representation based on clinical features such as bad habits, concomitant diseases, ongoing treatment,

tumor localization and histological characteristics, and macroscopic characteristics of the liver and bile ducts. Thus, in patients with intrahepatic tumor localization, there was a significant increase in the representation of bacteria from the genus *Clostridia UCG-014* (p = 0.023), while in patients with stage 3–4 of the disease, the content of bacteria of the genus *Odoribacter* significantly decreased (p = 0.012). Patients with anaplastic cancer confirmed by histologic examination had a significantly increased content of the genus *Saccharimonadaceae* (p = 0.029), unclassified genera from the family *Rhizobiaceae* (p = 0.036), and the genus *Faecalibacterium* (p = 0.043) compared to patients with other types of cancer. We also observed a significant increase in the abundance of bacteria from the genus *Agathobacter* in patients with dilated hepatic ducts (p = 0.022).

DISCUSSION

This study is the first to investigate the composition of the intestinal microbiota using 16S rRNA sequencing in a Russian population of patients with cholangiocarcinoma. In our study sample, intrahepatic localization of CCA was diagnosed in the majority of cases, more than half of the patients were diagnosed at TNM stages III-IV. The clinical symptoms corresponded to the course of the disease.

The study revealed that the composition of intestinal microbiota in patients with CCA in comparison with the control group is characterized by changes in the quantitative representation of individual microbial communities without significant differences in alpha- and beta-diversity.

The results of foreign studies demonstrated differences in the composition of the intestinal microbiota between patients with biliary tract cancer and healthy participants. However, there is currently no clear trend in changes in specific groups of microorganisms or a characteristic microbial profile in CCA, possibly due to differences in methodological approaches. It should be noted that our study revealed some modifications of microorganism representation comparable to the results of similar previous studies. Thus, in two studies, a change in the representation of the genus Bacteroides was noted in patients with CCA and hepatocellular carcinoma (HCC) [15, 16]. Bacteria of the genus *Bacteroides* are dominant representatives of the normal intestinal microbiota and perform various functions aimed at maintaining intestinal homeostasis [17]. However, certain species of the genus *Bacteroides*, such as *Bacteroides fragilis*, may play a role in the pathogenesis of various diseases and carcinogenesis [18, 19]. A study by Tuo Deng et al, 2022, also found an association with increased representation of the *unclassified Lachnospiraceae group NK4A136* [15].

Another study also noted an increase in *Parabacteroides* in intestinal microbiota samples from patients with hepatocellular carcinoma [20]. The gut microbiota of patients at early stages of HCC was characterized by a decrease in butyrate-producing bacteria and an increase in lipopolysaccharide-producing microorganisms compared to control group samples [20]. High levels of lipopolysaccharides have been shown to activate the NF-κB pathway, produce pro-inflammatory cytokines (TNFα, IL-6 and IL-1) and lead to inflammatory and oxidative damage to the liver, contributing to the development of hepatobiliary cancers [21–23].

The results of gut microbiota diversity indices assessment according to different studies are heterogeneous. A systematic review of studies investigating intestinal microbiota in patients with CCA showed that in most publications, the alpha-diversity index did not differ significantly from the controls, while two studies reported a decrease and in one study an increase in taxonomic diversity [24]. In a study of liver biopsy specimens, it was shown that samples from peritumor sites and HCC tissue had greater bacterial diversity compared to unaffected liver sites [25].

As a result of gut microbiota assessment using the balance selection method, we identified a number of bacteria that influence the change in the global balance of microorganisms in the samples. A microbiota profile with increased representation of *[Ruminococcus]_torques_group*, *Subdoligranulum*, *Parasutterella*, *unclassified Firmicutes* was characteristic of patients with CCA. In contrast, previous studies have noted a decrease in the representation of bacteria of the genus *Subdoligranulum* in patients with liver disease compared to healthy participants [26–28]. Bacteria of the genus *Parasutterella* are involved in bile acid metabolism [29].

The study of the gut microbiota is a challenging task due to the many internal and external factors affecting the composition of microbial communities, and it requires careful study design. The limitations

of our study include a small sample size and a single assessment of the microbiota in a stool sample.

CONCLUSION

This study revealed a number of significant differences in bacterial representation in patients with cholangiocarcinoma compared to control group participants. Considering the results of previous studies, microorganisms such as *Bacteroides* and *Lachnospiraceae_NK4A136_group* may be potential microbial markers of CCA development. Thus, further studies of the gut microbiota hold promise for the development of non-invasive tools for the early diagnosis of CCA.

REFERENCES

1. Banales J.M., Cardinale V., Carpino G., Marzioni M., Andersen J.B., Invernizzi, P. et al. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat. Rev. Gastroenterol. Hepatol*. 2016;13(5):261–280. DOI: 10.1038/nrgastro.2016.51
2. WHO's Global Health Estimates: Life expectancy and leading causes of death and disability. 2020. URL: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates
3. Fedorova O.S., Kovshirina Y.V., Kovshirina A.E., Fedotova M.M., Deev I.A., Petrovskiy F.I. et al. Opisthorchis felineus infection and cholangiocarcinoma in the Russian Federation: A review of medical statistics. *Parasitol. Int.* 2017;66(4):365–371. DOI: 10.1016/j.parint.2016.07.010.
4. Palmer W.C., Patel T. Are common factors involved in the pathogenesis of primary liver cancers? A meta-analysis of risk factors for intrahepatic cholangiocarcinoma. *J. Hepatol.* 2012;57(1):69–76. DOI: 10.1016/j.jhep.2012.02.022.
5. Uddin M.H., Li S., Jin Y., Choi M.H., Jang J.J., Hong S.T. C3H/He Mice as an Incompatible Cholangiocarcinoma Model by Clonorchis sinensis, Dicyclanil and N-Nitrosodimethylamine. *Korean J. Parasitol.* 2016;54(3):281–289. DOI: 10.3347/kjp.2016.54.3.281.
6. Woo H., Han J.K., Kim J.H., Hong S.T., Uddin M.H., Jang J.J. *In vivo* monitoring of development of cholangiocarcinoma induced with C. sinensis and N-nitrosodimethylamine in Syrian golen hamsters using ultrasonography and magnetic resonance imaging: a preliminary study. *Eur. Radiol*. 2017;27(4):1740–1747. DOI: 10.1007/s00330-016-4510-4.
7. Meng C., Bai C., Brown T.D., Hood L.E., Tian Q. Human gut microbiota and gastrointestinal cancer. *Genomics Proteomics Bioinformatics*. 2018;16(1):33–49. DOI: 10.1016/j.gpb.2017.06.002.
8. Adolph T.E., Grander C., Moschen A.R., Tilg H. Liver-microbiome axis in health and disease. *Trends Immunol*. 2018;39(9):712–723. DOI: 10.1016/j.it.2018.05.002.
9. Tang R., Wei Y., Li Y., Chen W., Chen H., Wang Q. et al. Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. *Gut.* 2018;67(3):534–541. DOI: 10.1136/gutjnl-2016-313332.
10. Ni J., Huang R., Zhou H., Xu X., Li Y., Cao P. et al. Analysis of the relationship between the degree of dysbiosis in gut microbiota and prognosis at different stages of primary hepatocellular carcinoma. *Front. Microbiol*. 2019;10:1458. DOI: 10.3389/fmicb.2019.01458.
11. Imhann F., Vich Vila A., Bonder M.J., Fu J., Gevers D., Visschedijk M.C. et al. Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. *Gut*. 2018;67(1):108–119. DOI: 10.1136/gutjnl-2016-312135.
12. Sripa B., Deenonpoe R., Brindley P.J. Co-infections with liver fluke and Helicobacter species: A paradigm change in pathogenesis of opisthorchiasis and cholangiocarcinoma? *Parasitol. Int*. 2017;66(4):383–389. DOI: 10.1016/j.parint.2016.11.016.
13. Chng K.R., Chan S.H., Ng A.H.Q., Li C., Jusakul A., Bertrand D. et al. Tissue microbiome profiling identifies an enrichment of specific enteric bacteria in *Opisthorchis viverrini* associated cholangiocarcinoma. *EBio Medicine*. 2016;8:195–202. DOI: 10.1016/j.ebiom.2016.04.034.
14. Sripa B., Bethony J.M., Sithithaworn P., Kaewkes S., Mairiang E., Loukas A. et al. Opisthorchiasis and opisthorchis-associated cholangiocarcinoma in Thailand and Laos. *Acta Trop.* 2011;120:S158–S168. DOI: 10.1016/j.actatropica.2010.07.006.
15. Deng T., Li J., He B., Chen B., Liu F., Chen Z. et al. Gut microbiome alteration as a diagnostic tool and associated with inflammatory response marker in primary liver cancer. *Hepatol. Int.* 2022;16(1):99–111. DOI: 10.1007/s12072-021-10279-3.
16. Zhang T., Zhang S., Jin C., Lin Z., Deng T., Xie X. et al. A Predictive model based on the gut microbiota improves the diagnostic effect in patients with cholangiocarcinoma. *Front. Cell Infect. Microbiol.* 2021;11:751795. DOI: 10.3389/fcimb.2021.751795.
17. Zafar H., Saier M.H. Jr. Gut bacteroides species in health and disease. *Gut. Microbes*. 2021;13(1):1–20. DOI: 10.1080/19490976.2020.1848158.
18. Bartolini I., Risaliti M., Ringressi M.N., Melli F., Nannini G., Amedei A. et al. Role of gut microbiota-immunity axis in patients undergoing surgery for colorectal cancer: Focus on short and long-term outcomes. *World J. Gastroenterol.* 2020;26(20):2498–2513. DOI: 10.3748/wjg.v26.i20.2498.
19. Mármol I., Sánchez-de-Diego C., Pradilla Dieste A., Cerrada E., Rodriguez Yoldi M.J. Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. *Int. J. Mol. Sci. 2017;18(1):197*. DOI: 10.3390/ijms18010197.
20. Ren Z., Li A., Jiang J., Zhou L., Yu Z., Lu H. et al. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. *Gut.* 2019;68(6):1014–1023. DOI: 10.1136/gutjnl-2017-315084.
21. Dapito D.H., Mencin A., Gwak G.Y., Pradere J.P., Jang M.K., Mederacke I. et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell.* 2012;21(4):504–516. DOI: 10.1016/j.ccr.2012.02.007.
22. Darnaud M., Faivre J., Moniaux N. Targeting gut flora to prevent progression of hepatocellular carcinoma. *J. Hepatol*. 2013;58(2):385–387. DOI: 10.1016/j.jhep.2012.08.019.

23. Nolan J.P. The role of intestinal endotoxin in liver injury: a long and evolving history. *Hepatology*. 2010;52(5):1829–1835. DOI: 10.1002/hep.23917.
24. Lederer A.K., Rasel H., Kohnert E., Kreutz C., Huber R., Badr M.T. et al. Gut Microbiota in Diagnosis, Therapy and Prognosis of Cholangiocarcinoma and Gallbladder Carcinoma-A Scoping Review. *Microorganisms.* 2023;11(9):2363. DOI: 10.3390/microorganisms11092363.
25. Huang J.H., Wang J., Chai X.Q., Li Z.C., Jiang Y.H., Li J. et al. The Intratumoral bacterial metataxonomic signature of hepatocellular carcinoma. *Microbiol. Spectr*. 2022;10(5):e0098322. DOI: 10.1128/spectrum.00983-22.
26. Bajaj J.S., Hylemon P.B., Ridlon J.M., Heuman D.M., Daita K., White M.B. et al. Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol*. 2012;303(6):G675–G685. DOI: 10.1152/ajpgi.00152.2012.
27. Qin N., Yang F., Li A., Prifti E., Chen Y., Shao L. et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature*. 2014;513(7516):59–64. DOI: 10.1038/nature13568.
28. Louis S., Tappu R.M., Damms-Machado A., Huson D.H., Bischoff S.C. Characterization of the gut microbial community of qbese patients following a weight-loss intervention using whole metagenome shotgun sequencing. *PLoS One*. 2016;11(2):e0149564. DOI: 10.1371/journal.pone.0149564.
29. Ju T., Kong J.Y., Stothard P., Willing B.P. Defining the role of *Parasutterella*, a previously uncharacterized member of the core gut microbiota. *ISME. J.* 2019;13:1520–1534. DOI: 10.1038/s41396-019-0364-5.

Authors' contribution

Fedorova O.S. – final approval of the manuscript for publication,. Kovshirina A.E. – substantiation of the manuscript, conception and design, analysis and interpretation of the data. Sokolova T.S. – conception and design,. Kulenich V.V. – analysis and interpretation of the data. Ogorodova L.M. – critical revision of the manuscript for important intellectual content.

Authors' information

Fedorova Olga S. – Dr. Sci. (Med.), Vice-Rector for Postgraduate Education and Research, Head of the Intermediate-Level Pediatrics Division with a Pediatric Diseases Course, General Medicine Department, Siberian State Medical University, Tomsk, olga.sergeevna.fedorova@gmail.com, https://orcid.org//0000-0002-7130-9609; +7 (3822)901-101(1506).

Kovshirina Anna E. – Teaching Assistant of the Propaedeutics of Internal Diseases Division with a Therapy Course, Department of Pediatrics, Siberian State Medical University, Tomsk, anna.evgenjevna.kovshirina@gmail.com, https://orcid.org/0000-0001-6116-8323.

Sokolova Tatiana S. – PhD, Associate Professor of the Intermediate-Level Pediatrics Division with a Pediatric Diseases Course, General Medicine Department, Siberian State Medical University, Tomsk, sokolova.ts@ssmu.ru, https://orcid.org/0000-0002-1085-0733.

Kulenich Victoria V. – Research Assistant of the Research and Educational Laboratory "Live Laboratory of Population-Based Studies", Siberian State Medical University, kulenich.vv@ssmu.ru, https://orcid.org/0009-0000-7416-5017.

Ogorodova Ludmila M. – Dr. Sci. (Med.), RAS Corresponding Member, Professor of the Intermediate-Level Pediatrics Division with a Pediatric Diseases Course, General Medicine Department, Siberian State Medical University, Tomsk, ogorodova.lm@ssmu.ru, https://orcid.org/0000-0002-2962-1076.

(✉) **Fedorova Olga S.,** olga.sergeevna.fedorova@gmail.

Received 15.10.2024;
approved after peer review 23.10.2024;
accepted 28.11.2024