Gut Dysbiosis and Hemodynamic Changes as Links of the Pathogenesis of Complications of Cirrhosis

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Abstract

**Background.** Hemodynamic changes (hyperdynamic circulation) and gut dysbiosis are observed in cirrhosis. It was suggested that gut dysbiosis contributes to the development of hyperdynamic circulation, which aggravates the course of cirrhosis. The aim is to test this hypothesis.

**Methods.** The cross-sectional observational study included 47 patients with cirrhosis. Stool microbiome was assessed using 16S rRNA gene sequencing. Echocardiography with a simultaneous assessment of blood pressure and heart rate was performed. Hemodynamic parameters were calculated.

**Results.** Hyperdynamic circulation was found in 34% of patients. Patients with hyperdynamic circulation had higher incidences of clinically significant ascites (p=0.018), overt hepatic encephalopathy (p=0.042), hypoalbuminemia (p=0.011), hypoprothrombinemia (p=0.019), systemic inflammation (p=0.002), and severe hyperbilirubinemia (p=0.042) than patients without hyperdynamic circulation. The abundance of Proteobacteria (p=0.012), *Enterobacteriaceae* (p=0.008), Bacilli (p=0.027), *Streptococcaceae* (p=0.044), *Lactobacillaceae* (p=0.034), *Enterococcaceae* (p=0.046), and Fusobacteria (p=0.026) increased and the abundance of Bacteroidetes (p=0.049) and Erysipelotrichia (p=0.029) decreased in the gut microbiome of patients with hyperdynamic circulation compared to patients without hyperdynamic circulation. The systemic vascular resistance value negatively correlated with the abundance of Proteobacteria (r=-0.423; p=0.003), *Enterobacteriaceae* (r=-0.417; p=0.004), and Fusobacteria (r=-0.401; p=0.005). Heart rate was negatively correlated with the abundance of Bacteroidetes (r=-0.453; p=0.001). The cardiac output value was positively correlated with the abundance of Proteobacteria (r=0.402; p=0.003), *Enterobacteriaceae* (r=0.424; p=0.003), Fusobacteria (r=0.281; p=0.049), and Bacilli (r=0.314; p=0.031), and negatively correlated with the abundance of Bacteroidetes (r=-0.313; p=0.032) and Erysipelotrichia (r=-0.329; p=0.024).

**Conclusion.** Gut dysbiosis is associated with hyperdynamic circulation, which is associated with a number of complications of cirrhosis.

Introduction

Hemodynamics changes in cirrhosis were described half a century ago and consist of arterial vasodilation (decreased systemic vascular resistance), hypotension, and increased cardiac output. This is defined as hyperdynamic circulation[1–3]. The study of experimental models of cirrhosis led to the hypothesis that these changes arise as a response to subclinical systemic inflammation which in turn is a consequence of bacterial translocation, the penetration of bacteria and their components from the intestinal contents into ascitic fluid, mesenteric lymph nodes, and portal and systemic blood flow[1–7]. Experts suggest that hyperdynamic circulation aggravates portal hypertension, creating a predisposition for the development of various complications of cirrhosis[3–7]. The main factors contributing to bacterial translocation are increased intestinal barrier permeability, small intestinal bacterial overgrowth (SIBO), and changes in the composition of the gut microbiota (gut dysbiosis)[4]. The relationship between SIBO,
hyperdynamic circulation, and complications of cirrhosis has been established\textsuperscript{[8–9]}. However, despite the intensive study of gut dysbiosis, no previous research has described its effect on systemic hemodynamics in cirrhosis\textsuperscript{[10–25]}. This study aimed to determine the relationship between gut dysbiosis and hemodynamic changes in cirrhosis as well as the relationship between these changes and the complications of this disease.

**Materials And Methods**

**Patients**

In this cross-sectional observational study, 100 consecutive patients with cirrhosis were admitted to the Department of Hepatology’s Clinic for Internal Diseases, Gastroenterology and Hepatology at Sechenov University (Moscow, Russia) and screened for inclusion. The study procedures were explained to potential participants, and written informed consent was obtained before enrollment. The present study was approved by the Ethics Committee of Sechenov University in accordance with the Declaration of Helsinki.

The inclusion criteria were as follows: diagnosis of cirrhosis verified by histology or clinical, biochemical, and ultrasound findings; and age between 18 and 70 years. The exclusion criteria were as follows: use of lactulose, lactitol, or other prebiotics, probiotics, antibiotics, or metformin in the past 6 weeks; alcohol consumption in the past 6 weeks; or inflammatory bowel disease, cancer, or any other serious disease. Of the original 100 patients screened for inclusion, 47 met the criteria and were enrolled in the study (Fig. 1).

**Gut microbiome analysis**

The morning after admission, a stool sample was taken into a sterile disposable container and immediately frozen at \(-80^\circ\text{C}\)\textsuperscript{[26]}. DNA from the stool was isolated using the MagNa Pure Compact Nucleic Acid Isolation Kit I (Roche, Basel, Switzerland) according to the manufacturer's instructions. Libraries for sequencing were prepared by two rounds of PCR amplification. In the first round, specific primers for the v3-v4 region of the 16S ribosomal RNA gene were used:

\[
16S\text{-}F \text{TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG} \text{and } 16S\text{-}R \text{GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC.}
\]

After amplification, the PCR product was purified using AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). Then, a second round of PCR was performed to attach specific adapters and enable multiplexing of the samples. To begin, 5 µL of the first PCR product was added to the reaction after ball cleaning with primers containing Illumina indices (Nextera XT Index v2 Primers; San Diego, CA, USA) and adapter sequences as well as 2× KAPA HiFi HotStart ReadyMix. The amplification products were also purified using AMPure XP beads (Beckman Coulter). The concentrations of the prepared libraries were then measured using a Qubit 2.0 fluorimeter (London, UK) and quantitative PCR. The quality of the libraries was
assessed using the Agilent 2100 Bioanalyzer (Santa Clara, CA, USA). The libraries were mixed in equal proportions and diluted to the required concentration to be run on a MiSeq (Illumina) device. Pair-end readings of 300 + 300 nucleotides were obtained. Reads were trimmed from the 3′-tail with Trimmomatic (Illumina) and then merged into a single amplicon with the MeFiT tool\cite{27-28}. We did not perform operational taxonomic unit picking; instead, we classified amplicon sequences with the Ribosomal Database Project (RDP) classifier and RDP database\cite{29}.

**Systemic hemodynamic assessment**

Echocardiography was performed at rest according to the guidelines of the American Society of Echocardiography\cite{30-33}. The systolic and diastolic blood pressure and heart rate were measured using an automatic oscillometric sphygmomanometer (AND, Japan) simultaneously with the assessment of the stroke volume. Calculations of hemodynamic parameters are presented in Table 1\cite{30-35}.

| Parameter                                           | Calculation                                                                 |
|-----------------------------------------------------|----------------------------------------------------------------------------|
| End-diastolic and end-systolic volume of the left ventricle | Modified Simpson's disk method                                              |
| Ejection fraction of the left ventricle              | \((\text{end-diastolic volume})-(\text{end-systolic volume})/(\text{end-diastolic volume})\) |
| Stroke volume                                       | \((\text{Doppler velocity time integral})\times(\text{cross-sectional aorta area})^{34}\) |
| Mean arterial pressure                              | \(((\text{systolic blood pressure}) + 2\times(\text{diastolic blood pressure})) / 3\) |
| Cardiac output                                      | \((\text{stroke volume})\times(\text{heart rate})\)                          |
| Systemic vascular resistance                        | \((\text{mean arterial pressure}) / (\text{cardiac output})\)                |
| Systolic pulmonary artery pressure                  | \((\text{right atrium pressure estimated from diameter of inferior vena cava and respiratory changes}) + 4\times(\text{the peak velocity of the tricuspid valve regurgitant jet})^2^{32-33}\) |
| Mean pulmonary artery pressure                      | 0.61\times(\text{systolic pulmonary artery pressure}) + 2 \text{ mmHg}^{35}\) |

The criterion for portopulmonary hypertension was a combination of the presence of signs of portal hypertension and mean pulmonary artery pressure above 25 mm Hg\cite{36}.

No generally accepted criteria for hyperdynamic circulation are available. Therefore, we diagnosed a patient with this disorder if their cardiac output was greater than the mean + 2 standard deviations (5.5 L/min) of healthy individuals examined in the same way during the check-up. The control group \((n = 50)\) did not significantly differ from the patients with cirrhosis in terms of age and gender distribution.
Statistical analysis

Statistical analysis was performed with STATISTICA 10 (StatSoft Inc., Tulsa, OK, USA) and SPSS Statistics (IBM SPSS, Armonk, NY, USA) software. The data are presented as medians[interquartile ranges]. The abundance of taxa of the gut microbiota is presented as a percentage. Differences between continuous variables were assessed with the Mann-Whitney test because many variables were not distributed normally. Fisher’s exact test was used to assess the differences between categorical variables. Correlations between variables were computed using Spearman’s rank correlation. P-values ≤ 0.05 were considered as statistically significant.

Results

Hyperdynamic circulation was found in 16/47 (34.0%) patients, including 2/19 (10.5%) patients with Child-Pugh class A, 8/18 (44.4% vs. 10.5%; p = 0.024) patients with Child-Pugh class B, and 6/10 (60.0% vs. 10.5%; p = 0.002) patients with Child-Pugh class C.

Patients with hyperdynamic circulation had more severe cirrhosis according to the Child-Pugh scale, lower albumin and prothrombin levels, higher C-reactive protein and total bilirubin levels in the blood, lower systemic vascular resistance, higher left ventricular end-diastolic and stroke volumes, and higher incidences of portopulmonary hypertension, clinically significant ascites (grade 2 and 3 ascites according to the classification of the International Club of Ascites), overt hepatic encephalopathy, hypoalbuminemia, hypoprothrombinemia, systemic inflammation, and severe hyperbilirubinemia than patients without hyperdynamic circulation. No significant difference between the groups of patients was observed for incidences of minimal ascites (grade 1 ascites according to the classification of the International Club of Ascites), mild hyperbilirubinemia, minimal hepatic encephalopathy, and esophageal varices, spleen size, main parameters of complete blood count, heart rate, mean blood pressure, left ventricular ejection fraction, and serum creatinine level. The difference between the groups of patients in mean pulmonary artery pressure, serum sodium and potassium levels almost reached the limit of significance (Table 2).
|                             | Cirrhosis with hyperdynamic circulation (n = 16) | Cirrhosis without hyperdynamic circulation (n = 31) | p       |
|-----------------------------|------------------------------------------------|------------------------------------------------|---------|
| Age, years                  | 48.5[38.5–53.0]                                | 51.0[37.0–60.0]                                | 0.605   |
| Body mass index, kg/m²      | 24.6[22.8–29.3]                                | 23.5[22.3–27.5]                                | 0.307   |
| Male/female                 | 9/7                                            | 13/18                                          | 0.266   |
| Etiology of cirrhosis: alcohol | 6 (37.5%)                                    | 7 (22.6%)                                     | >0.050  |
| autoimmune hepatitis        | 2 (12.5%)                                     | 5 (16.2%)                                     |         |
| HBV                         | 1 (6.3%)                                      | 5 (16.1%)                                     |         |
| HCV                         | 2 (12.5%)                                     | 7 (22.6%)                                     |         |
| mixed                       | 4 (25.0%)                                     | 5 (16.1%)                                     |         |
| cryptogenic                 | 1 (6.3%)                                      | 2 (6.5%)                                      |         |
| Child–Pugh score            | 9[8–11]                                       | 6[6–8]                                        | 0.003   |
| End-diastolic volume of the left ventricle, mL | 132[120–142]                                | 97[87–107]                                    | <0.001  |
| Ejection fraction of the left ventricle, % | 62.2[60.1–64.4]                              | 60.8[58.7–63.8]                               | 0.296   |
| Stroke volume, mL           | 80[75–86]                                     | 59[55–65]                                     | <0.001  |
| Heart rate, bpm             | 76[67–84]                                     | 71[65–81]                                     | 0.393   |
| Cardiac output, L/min       | 5.8[5.6–6.7]                                  | 4.2[3.8–4.6]                                  | <0.001  |
| Mean blood pressure, mmHg   | 83[78–92]                                     | 88[78–95]                                     | 0.875   |
| Systemic vascular resistance, dyn·s·cm⁻⁵ | 1095[1011–1302]                             | 1640[1436–1917]                               | <0.001  |
| Mean pulmonary artery pressure, mmHg | 20.3[17.3–26.4]                           | 17.3[13.6–20.3]                               | 0.075   |
| Portopulmonary hypertension, n (%) | 5 (12.8%)                                    | 1 (3.2%)                                      | 0.013   |
| Esophageal varices (Grade 1), n (%) | 4 (25.5%)                                    | 11 (35.5%)                                    | 0.349   |
| Esophageal varices (Grade 2–3), n (%) | 7 (43.8%)                                    | 16 (51.6%)                                    | 0.420   |
|                                    | Cirrhosis with hyperdynamic circulation (n = 16) | Cirrhosis without hyperdynamic circulation (n = 31) | p   |
|------------------------------------|-----------------------------------------------|---------------------------------------------------|-----|
| Minimal hepatic encephalopathy, n (%) | 4 (25.0%)                                      | 14 (51.2%)                                       | 0.151 |
| Overt hepatic encephalopathy, n (%) | 9 (56.3%)                                      | 8 (23.8%)                                        | 0.042 |
| Ascites, n (%)                     | 11 (68.8%)                                     | 15 (48.4%)                                       | 0.154 |
| Minimal ascites, n (%)             | 3 (18.8%)                                      | 10 (32.3%)                                       | 0.266 |
| Clinically significant ascites, n (%) | 8 (50.0%)                                     | 5 (16.1%)                                        | 0.018 |
| Red blood cells, 10^{12} cell/L   | 3.7[3.1–4.2]                                   | 3.9[3.6–4.5]                                     | 0.092 |
| White blood cells, 10^9 cell/L     | 4.5[3.4–7.3]                                   | 3.5[2.8–4.8]                                     | 0.127 |
| Platelets, 10^9 cell/L             | 83[59–107]                                     | 81[58–115]                                       | 0.955 |
| Serum total protein, g/L           | 69[63–76]                                      | 73[64–78]                                        | 0.406 |
| Serum albumin, g/L                 | 31[28–36]                                      | 38[32–42]                                        | 0.012 |
| Hypoalbuminemia (serum albumin < 35 g/L), n (%) | 11 (68.8%)                                     | 9 (29.0%)                                        | 0.011 |
| Serum total bilirubin, μmol/L      | 56[40–83]                                      | 31[22–55]                                        | 0.007 |
| Mild hyperbilirubinemia (total bilirubin = 22–51 μmol/L), n (%) | 5 (31.2%)                                      | 16 (51.6%)                                       | 0.154 |
| Severe hyperbilirubinemia (total bilirubin > 51 μmol/L), n (%) | 9 (56.3%)                                      | 8 (25.8%)                                        | 0.042 |
| Prothrombin index (Quick test), %  | 52[45–62]                                      | 66[57–71]                                        | 0.001 |
| Hypoprothrombinemia (prothrombin index < 60%), n (%) | 11 (68.8%)                                     | 10 (32.3%)                                       | 0.019 |
| Creatinine, mg/dL                  | 0.78[0.57–0.91]                                | 0.70[0.62–0.90]                                  | 0.875 |
| Serum sodium, mmol/L               | 140[138–141]                                   | 141[140–144]                                     | 0.081 |
| Serum potassium, mmol/L            | 4.2[3.2–4.6]                                   | 4.4[4.1–4.7]                                     | 0.072 |
| Serum glucose, mmol/L              | 5.2[4.7–5.6]                                   | 5.2[4.6–5.7]                                     | 0.946 |
| Alanine aminotransferase, U/L      | 48[26–127]                                     | 36[23–61]                                        | 0.225 |
| Aspartate aminotransferase, U/L     | 77[49–176]                                     | 44[29–64]                                        | 0.018 |
| Gamma glutamyl transferase, U/L     | 101[37–166]                                    | 59[27–122]                                       | 0.795 |
|                                      | Cirrhosis with hyperdynamic circulation (n = 16) | Cirrhosis without hyperdynamic circulation (n = 31) | p    |
|--------------------------------------|-------------------------------------------------|---------------------------------------------------|------|
| Alkaline phosphatase, U/L             | 223[176–301]                                    | 222[166–310]                                      | 0.893|
| C-reactive protein, mg/L              | 13.8[8.9–17.1]                                  | 2.3[0.5–9.2]                                      | 0.002|
| Systemic inflammation (C-reactive protein > 10 mg/L), n (%) | 10 (62.5%)                                      | 4 (12.9%)                                         | 0.001|
| Splenic length, cm                    | 15.3[14.0–16.5]                                 | 15.7[13.8–19.2]                                  | 0.718|

None of the included patients had spontaneous bacterial peritonitis, hepatopulmonary or hepatorenal syndrome.

The abundance of Proteobacteria, *Enterobacteriaceae*, Bacilli, *Streptococcaceae*, *Lactobacillaceae*, *Enterococccae*, and Fusobacteria were increased and the abundance of Bacteroidetes and Erysipelotrichia were decreased in the gut microbiome of patients with hyperdynamic circulation compared to patients without hyperdynamic circulation (Table 3, Fig. 2).
Table 3
Comparison of the gut microbiome at different taxonomic levels between patients with and without hyperdynamic circulation

| Taxa                        | Cirrhosis with hyperdynamic circulation (n = 16) | Cirrhosis without hyperdynamic circulation (n = 31) | p   |
|-----------------------------|-----------------------------------------------|--------------------------------------------------|-----|
| **Firmicutes**              | 83.7[68.4–91.1]                               | 85.7[69.3–91.0]                                   | 0.920 |
| **Clostridia**              | 68.5[52.6–83.8]                               | 76.9[62.4–83.2]                                   | 0.375 |
| **Lachnospiraceae**         | 35.4[22.8–52.7]                               | 36.5[23.6–47.5]                                   | 0.902 |
| **Ruminococcaceae**         | 17.0[9.7–34.1]                                | 23.7[15.2–36.1]                                   | 0.466 |
| **Peptostreptococcaceae**   | 0.70[0.15–3.50]                               | 0.22[0.04–0.87]                                   | 0.148 |
| **Clostridiaceae**          | 0.44[0.07–1.59]                               | 0.10[0.01–0.47]                                   | 0.145 |
| **Bacilli**                 | 6.65[1.53–14.5]                               | 0.91[0.40–4.61]                                   | 0.027 |
| **Streptococcaceae**        | 2.48[0.42–10.4]                               | 0.44[0.13–3.15]                                   | 0.044 |
| **Lactobacillaceae**        | 0.34[0.07–1.16]                               | 0.11[0.01–0.38]                                   | 0.034 |
| **Enterococcaceae**         | 0.04[0.00-0.09]                               | 0.00[0.00-0.04]                                   | 0.046 |
| **Negativicutes**           | 0.48[0.22–0.78]                               | 0.41[0.08–1.27]                                   | 0.849 |
| **Veillonellaceae**         | 0.47[0.11–0.76]                               | 0.17[0.02–0.87]                                   | 0.296 |
| **Erysipelotrichia**        | 0.28[0.09–0.38]                               | 0.65[0.29–1.30]                                   | 0.029 |
| **Fusobacteria**            | 0.01[0.00-0.09]                               | 0.00[0.00-0.01]                                   | 0.026 |
| **Bacteroidetes**           | 5.59[1.29–6.37]                               | 6.93[3.26–15.7]                                   | 0.049 |
| **Bacteroidaceae**          | 1.15[0.17–3.31]                               | 2.22[0.81–4.28]                                   | 0.135 |
| **Rikenellaceae**           | 0.30[0.01–1.01]                               | 0.30[0.05–0.88]                                   | 0.508 |
| **Porphyromonadaceae**      | 0.22[0.02–0.42]                               | 0.29[0.08–0.53]                                   | 0.204 |
| **Prevotellaceae**          | 0.08[0.01–2.71]                               | 0.27[0.01–3.11]                                   | 0.400 |
| **Actinobacteria**          | 0.72[0.44–1.37]                               | 0.79[0.25–3.06]                                   | 0.884 |
| **Bifidobacteriaceae**      | 0.44[0.14–1.09]                               | 0.56[0.04–2.17]                                   | 0.973 |
| **Proteobacteria**          | 2.40[1.16–8.21]                               | 0.65[0.12–2.60]                                   | 0.012 |
| **Enterobacteriaceae**      | 2.02[0.56–7.63]                               | 0.37[0.02–1.75]                                   | 0.008 |
| **Verrucomicrobiae**        | 0.01[0.00-1.40]                               | 0.01[0.00-0.85]                                   | 0.329 |
| **Akkermansiaceae**         | 0.01[0.00-1.39]                               | 0.00[0.00-0.27]                                   | 0.222 |
The systemic vascular resistance value negatively correlated with the abundance of Proteobacteria, *Enterobacteriaceae*, and Fusobacteria. This correlation with the abundance of Bacilli almost reached the limit of significance. Left ventricular end-diastolic volume positively correlated with the abundance of Proteobacteria, *Enterobacteriaceae*, and Fusobacteria. Its negative correlation with the abundance of Erysipelotrichia almost reached the limit of significance. Heart rate was negatively correlated with the abundance of Bacteroidetes. The cardiac output value was positively correlated with the abundance of Proteobacteria, *Enterobacteriaceae*, Fusobacteria, and Bacilli, and negatively correlated with the abundance of Bacteroidetes and Erysipelotrichia. No correlation was observed between the abundance of the main taxa of gut microbiome and mean blood pressure, ejection fraction, and mean pulmonary artery pressure values (Table 4).
Table 4
Correlation matrix of the main taxa of the gut microbiome and the main hemodynamic parameters in cirrhosis

|                  | EDV  | EF   | SV   | HR   | CO   | MBP  | SVR  | MPAP |
|------------------|------|------|------|------|------|------|------|------|
| Clostridia       | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. |
| Bacilli          | N.S. | N.S. | N.S. | N.S. | r = 0.314 | N.S. | r = -0.251 | N.S. |
|                  |      |      |      |      |      |      |      |      |
|                  |      |      |      |      |      |      |      |      |
| Erysipelotrichia | r = -0.251 | N.S. | r = -0.288 | N.S. | r = -0.329 | N.S. | N.S. | N.S. |
|                  | p = 0.088 |      | p = 0.049 |      | p = 0.024 |      |      |      |
| Fusobacteria     | r = 0.291 | N.S. | N.S. | N.S. | r = 0.281 | N.S. | r = -0.401 | N.S. |
|                  | p = 0.048 |      |      |      | p = 0.049 |      | p = 0.005 |      |
| Bacteroidetes    | N.S. | N.S. | N.S. | r = -0.453 | r = -0.313 | N.S. | N.S. | N.S. |
|                  |      |      |      |      |      |      |      |      |
|                  |      |      |      |      |      |      |      |      |
| Proteobacteria   | r = 0.396 | N.S. | r = 0.373 | N.S. | r = 0.402 | N.S. | r = -0.423 | N.S. |
|                  | p = 0.006 |      | p = 0.010 |      | p = 0.005 |      | p = 0.003 |      |
| Enterobacteriaceae | r = 0.370 | N.S. | r = 0.348 | N.S. | r = 0.424 | N.S. | r = -0.417 | N.S. |
|                  | p = 0.011 |      | p = 0.016 |      | p = 0.003 |      | p = 0.004 |      |

CO – Cardiac output; EDV– End-diastolic volume of the left ventricle; EF – Ejection fraction of the left ventricle; HR – Heart rate; MBP – Mean blood pressure; MPAP – Mean pulmonary artery pressure; N.S. – Not significant; SV – Stroke volume; SVR – Systemic vascular resistance.

Discussion

Hyperdynamic circulation was observed in one-third of patients with cirrhosis, and the frequency of its detection increased with an increase in the Child-Pugh cirrhosis class. The increase in cardiac output was accompanied by a decrease in systemic vascular resistance, with no significant decrease in blood pressure. That is, the state of systemic hemodynamics in most of our patients was compensated by fluid retention and increased heart function, neutralizing the hypotonic effect of systemic vasodilation. The increased cardiac output was due to an increase in venous return to the heart, which led to an increase in
end-diastolic volume. Heart rate and ejection fraction, which are other factors that could increase cardiac output, did not significantly differ between the groups of patients with and without hyperdynamic circulation, indicating their insignificant influence on its development. This is consistent with the underfilling hypothesis, which considers vasodilation as a primary disorder, and fluid retention and increased venous return to the heart with an increase in cardiac output as secondary changes\textsuperscript{[2–4]}.

Notably, an increase in end-diastolic volume is usually characteristic of systolic heart failure, but it is not associated with a decrease in ejection fraction in patients with cirrhosis\textsuperscript{[9]}. Moreover, the serum level of that biomarker of heart failure as N-terminal brain natriuretic peptide does not depend on ejection fraction, but is associated, on the contrary, with increased heart function in these patients\textsuperscript{[37]}.

Complications of cirrhosis were differently associated with hyperdynamic circulation in our study. Some of them (hypoalbuminemia, hypoprothrombinemia, systemic inflammation, portopulmonary hypertension) were more often in patients with this disorder than in those without it. The presence of others (esophageal varices) was not associated with it. The association of hyperdynamic circulation with complications of cirrhosis from the third group (ascites, hyperbilirubinemia, hepatic encephalopathy) depended on their severity: it was absent in their mild and minimal forms, but their severe forms were associated with it. This may be considered as confirmation of the hypothesis that increased cardiac output aggravates the course of portal hypertension but is not its primary cause. Moreover, our study was cross-sectional and it is not entirely correct to judge causal relationships. The primary question here was whether decreased liver function led to the development of hyperdynamic circulation, whether hyperdynamic circulation worsened liver function, or whether they both exacerbated each other, leading to a vicious circle. Additional studies are required to determine the changes in liver function in patients with the same level of decreased liver function, depending on the presence or absence of hyperdynamic circulation. The incidence of hyperdynamic circulation development should be prospectively investigated and compared between patients with varying degrees of compensation for liver function in the other group of future studies.

Unfortunately, we could not measure the hepatic venous pressure gradient, which is considered to be the main quantitative characteristic of portal hypertension\textsuperscript{[38]}.

Our study is the first to assess the relationship between gut dysbiosis and hemodynamic changes in cirrhosis. Despite disagreements between the results of several previous studies, most indicated that the abundance of bacteria under the Proteobacteria phylum\textsuperscript{[10–18,21–23]}, which contains active endotoxin, and Bacilli class\textsuperscript{[13–23]}, which are capable of bacterial translocation, increase in the gut microbiome with cirrhosis. Thus, an increase in the abundance of these bacteria can be considered a biomarker of gut dysbiosis in cirrhosis. These bacteria are responsible for molecular (endotoxin) and cellular bacterial translocation in cirrhosis\textsuperscript{[39]}.

In this study, the abundance of Bacilli and Proteobacteria increased in patients with hyperdynamic circulation and correlated with the values of the main markers of hyperdynamic circulation, namely systemic vascular resistance and cardiac output. This may support the hypothesis that bacterial
translocation of these bacteria and their components leads to vasodilation and hyperdynamic circulation. A similar relationship is also established for the minor taxon Fusobacteria, which also contain endotoxins. Only one article\textsuperscript{22} reported an increase in the content of these bacteria in the gut microbiome in cirrhosis. This may be due to their low abundance in the gut microbiome, so these bacteria do not attract the attention of researchers.

An interesting finding was the decrease in Bacteroidetes abundance in patients with hyperdynamic circulation, considering these bacteria also have endotoxins. The abundance of these bacteria does not correlate with the degree of vasodilation but is associated with a decrease in heart rate, which can prevent the development of hyperdynamic circulation. The mechanism by which Bacteroidetes affect the heart rate is not clear. It seems that the presence of endotoxin is not an indicator of bacterial pathogenicity and its ability to translocate. It should be remembered that Bacteroidetes, together with bacteria under the Clostridia class, are the main taxa of normal human microbiota, and changes in their abundance in cirrhosis compared with healthy individuals are reported differently in different publications. Bacteroidetes abundance either increases\textsuperscript{11,24}, decreases\textsuperscript{10,19,22}, does not change\textsuperscript{21}, or changes depending on the state of liver function\textsuperscript{16} in cirrhosis. Bacteroidetes showed a protective effect against hyperdynamic circulation in our study.

The abundance of beneficial bacteria under the Clostridia class in the gut microbiome does not significantly differ between patients with and without hyperdynamic circulation and does not correlate with any of the hemodynamic parameters in cirrhosis.

An unexpected finding was the negative correlation between markers of hyperdynamic circulation and the abundance of Erysipelotrichia that are a minor class under the Firmicutes phylum. Among the 4 main classes under this phylum, it is the least studied and might be underestimated by researchers.

Changes in the gut microbiome in hemodynamic circulation mainly signifies a redistribution of the proportion of bacteria containing endotoxins, where Proteobacteria and Fusobacteria that have active endotoxins replace Bacteroidetes that have weak endotoxins\textsuperscript{40} (Fig. 2).

Probiotics, which are living bacteria used for dysbiosis, showed their effects on hemodynamic parameters in cirrhosis in small uncontrolled studies, which require randomized controlled trials to confirm\textsuperscript{41}.

Our study is the first to confirm that gut dysbiosis is associated with hemodynamic changes in cirrhosis. We further showed that the presence of these changes is associated with a number of complications of cirrhosis. Thus, hemodynamic changes may be considered a pathogenetic link between gut dysbiosis and these complications of cirrhosis. However, this hypothesis requires verification in further prospective studies, the ideas of which we also proposed. All of these contribute to the strength of our study.

The limitation of our study is its small sample size, although this did not prevent us from obtaining significant results.
In conclusion, we have shown that gut dysbiosis is associated with hyperdynamic circulation, which in turn is associated with a number of complications of cirrhosis.

**Declarations**

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**Conflicts of interest:** No.

**Ethics approval and Consent to participate:** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

**Consent for publication:** Not applicable.

**Availability of data and material:** The data can be provided upon request to the corresponding author.

**Code availability:** Not applicable.

**Author contributions:** Research idea - Vladimir Ivashkin. Study design - Vladimir Ivashkin and Roman Maslennikov. Research and data analysis - all authors. Draft writing - Roman Maslennikov. Draft editing - all authors. Roman Maslennikov is the guarantor.

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**Figures**

**Figure 1**

CONSORT 2010 Flow Diagram
Figure 2

The composition of the gut microbiome in the patients with and without hyperdynamic circulation