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Gut microbiota and systemic inflammation in patients with chronic heart failure

Aim	To study the interrelationship between intensity of chronic systemic inflammation (CSI) with severity of the condition and intestinal microbiocenosis parameters in patients with chronic heart failure (CHF).
Material and methods	47 hospitalized patients with symptomatic CHF were evaluated. The following parameters were determined: clinical condition; N-terminal pro-B-type natriuretic peptide (NT-proBNP). C-reactive protein (CRP); serum interleukins (IL) 6 and 10; and intestinal microbiocenosis composition by mass-spectrometry of microbial markers in whole blood. Microbiocenosis indexes were compared in the main group and in 38 outpatient patients with arterial hypertension and ischemic heart disease without CHF.
Results	Direct, medium-power correlations were found between CRP and IL-6 concentrations and severity of clinical condition (NT-proBNP, XCH stage, and edema severity) in patients with CHF. Most patients with CHF had lower numbers of bifido-, lacto-, propionic-, and eubacteria, and *Clostridium (C.) ramosum* and higher numbers of aspergillus. Among CHF patients, the highest indexes of endotoxemia, gram (-) bacteria, cocci, actinomycetes, and microfungi were observed in the group with NT-proBNP from 400 to 2000 pg/ml. Direct correlations were observed for amounts of *C. hystolyticum, Pseudonocardia spp.,* and *Aspergillus spp*. with IL-6 and IL-10 and unidirectional inverse correlation were observed for these cytokines with *Propionibacterium acnes* and jensenii, *Streptomyces spp.,* and *Nocardia asteroides.* In addition, IL-6 concentration was negatively correlated with contents of *Staphylococcus aureus, C. difficile, C. ramosum, Eggerthella lenta,* and *Corynebacterium spp.* and was positively correlated with *C. propionicum, Moraxella spp.* and *Flavobacterium spp.* Concentration of IL-6 directly correlated with the number of *Eubacterium spp.* and inversely correlated with numbers of *Ruminicoccus spp.* and *Streptomyces farmamarensis*. The amount of *Streptomyces farmamarensis* negatively correlated with CRP concentrations.
Conclusion	The study results evidence the significance of intestinal microbial-tissue complex in the pathogenesis of CSI in CHF and allow suggesting this complex as a promising target for therapy.

Keywords	Chronic heart failure; intestinal microbiota; systemic inflammation; lipopolysaccharide; microbial markers; interleukin
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Chronic systemic inflammation (CSI) is a key to the pathogenesis of many diseases. It plays a special role in the progression of chronic heart failure (CHF). Although several papers [1–3] demonstrate the importance of neurohumoral factors and cytokines (CK) in CHF-associated cardiac remodeling, specific mechanisms are still under discussion. The intestinal microbiota has been studied as a key element of CSI initiation in patients with CHF [4–9]. Intestinal endotoxin, i.e., gram-negative [gram (-)] bacterial lipopolysaccharide [LPS]), is the most common proinflammatory factor [4, 6]. Moreover, an entire group of fecal microorganisms is involved in the formation of CHF-associated inflammatory responses [7, 8]. However, the evidence obtained is ambiguous. Several authors [10, 11] did not report any changes in the levels of CSI factors, i.e., tumor necrosis factor-α [TNF-α], interleukin 6 [IL-6] and blood endotoxin, or the number of intestinal bifidobacteria and lactobacilli in patients with CHF.

The objective of the present study was to evaluate the correlation between CSI intensity, the severity of the condition, and intestinal microbiocenosis parameters in patients with CHF. The study is relevant due its controversial findings and the implementation of advanced methods for intestinal microbiocenosis evaluation.

Material and methods

Forty-seven patients (38 males/9 females) with symptomatic CHF who had been admitted to the cardiology inpatient unit of the Kirov Military Medical Academy were enrolled in a cross-sectional, analytical, controlled trial. The study was approved

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by the Ethics Committee, and all patients signed voluntary, informed consent forms.

The following were study exclusion criteria: 1) age >80 years, 2) a history of acute and chronic diseases within six months after an exacerbation, including myocardial infarction, cerebrovascular accident, pulmonary embolism, chronic renal failure (glomerular filtration rate <45 ml/min/1.73 m2 or proteinuria >1 g/day), 3) mental incapacity, 4) hepatic cirrhosis [except cardiac form], 5) malignant neoplasms regardless of localization, 6) tuberculosis, 7) systemic connective tissue diseases, 8) diabetes mellitus (glycohemoglobin level >6.5%), or 9)consent withdrawal regardless of the trial phase. The median age of patients was 63 (58.5; 66) years. The median duration of CHF was 24 (9; 78) months. Males with functional class (FC) III-IV CHF predominated among patients. Most patients demonstrated signs of maladaptive cardiac remodeling, reduced left ventricular ejection fraction, and high systolic pulmonary pressure (Table 1). CHF was caused by coronary artery disease (CAD) (n=36 [77%]), hypertension (n=35 [75%]), and their combination (n=24 [51%]). Twenty-two (47%) patients had a history of myocardial infarction. One (2%) and six (13%) patients had hypertrophic and dilated cardiomyopathies, respectively. Thirty four (72%) patients had atrial fibrillation, including permanent form (n=24 [51%]) or persistent and paroxysmal forms (n=10 [21%]).

Patients underwent routine examination and treatment of CHF according to current guidelines. Routine laboratory and instrumental tests and transthoracic echocardiography (VIVID 3, Israel) were performed. The clinical assessment scale adapted by V.Yu. Mareev (SHOKS) and a 6-min walk test were used to evaluate CHF FC. The severity of swelling was evaluated semi-quantitatively: 0=absence, 1=pastosity, 2=edemas, 3=anasarca. The severity of pulmonary congestion was evaluated semi-quantitatively based on radiographic and lung auscultation findings: 0=no radiographic and auscultatory signs of congestion, 1=radiographic findings of congestion and/or congestive rales at the lower lung level (below the 8th rib along the scapular line), 2=unilateral hydrothorax and/or congestive rales under scapulae (inferior angle of the scapula), 3=bilateral hydrothorax and/or congestive rales over the entire lung surface. The duration of the current worsening of CHF was expressed in days before admission from the onset/progression of symptoms. Decompensated CHF was described as a progression requiring the administration of diuretics or an increase in the doses. At the time of admission, 70% and 45% of patients needed intravenous loop diuretics and inotropics, respectively. Since the beginning of the therapy, all patients received angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers, and in addition 81% received beta-blockers, and 90% received spironolactone.

N-terminal pro-brain natriuretic peptide (NT-proBNP), C-reactive protein (CRP), interleukin-10 (IL-10), and IL-6 levels were evaluated by ELISA in serum samples collected within the first 24 hours after admission (Vector Best Kits, Russia). Blood levels of higher fatty acids, aldehydes, and alcohols specific for particular intestinal microorganisms were evaluated by mass spectrometry of microbial markers. Based on these findings, assays of 54 species (geni) of bacteria and fungi in the parietal layer were performed, and endotoxinemia levels were estimated [12]. These findings were compared with data of a sample of 38 outpatients comparable by sex and age with hypertension and with chronic CHF-free CAD.

Patients were divided into three groups depending on the levels of serum NT-proBNP to evaluate the intensity of CSI and endoecologycal parameters in accordance with the severity of the patient's conditions (Table 1). This approach was applied by Gheorghiade et al. (2010). We believe that it clearly shows a correlation between neurohumoral and inflammatory activation in patients with CHF [13]. Group 1 included 9 patients with NT-proBNP<400 pg/ml, Groups 2 and 3 included subjects with NT-proBNP levels corresponding to moderate and severe decompensated CHF (400–2000 pg/ml and >2000 pg/ml, respectively). These groups were comparable in sex and age composition.

Data were processed with Microsoft Excel 2007 and Statistica for Windows (trial version 13.0). Normally distributed values of test parameters in each group, as shown by the Shapiro-Wilk W-test, were expressed as the mean (M) and standard deviation (σ). Non-normal distributed values were expressed as the median (Me) and interquartile range (Q25; Q75). These groups were compared using the Kruskal-Wallis rank analysis of variance and pairwise comparison using the Mann-Whitney test with Bonferroni correction. Spearman's or Kendall's rank correlation coefficients (τ) were calculated to evaluate the nature and strength of the correlation between variables. The critical significance point was $p<0.05$.

Results

Significant direct correlations were found between the levels of CRP and IL-6 and major clinical severity parameters (Table 2). Levels of NT-proBNP, CHF stage, and severity of swelling most closely correlated with the content of pro-inflammatory factors. There was a moderate direct correlation between levels of CRP and the duration of worsening conditions of patients.

An increase in NT-proBNP levels was associated with higher concentrations of CRP, IL-6 and IL-10 (Table 3). Direct correlations were detected between IL-10 and NT-proBNP levels ($\tau=0.21$, $p=0.039$), IL-6 and CRP levels ($\tau=0.48$, $p=0.0007$), IL-6 and IL-10 levels ($\tau=0.43$, $p=0.003$).

Most patients with CHF demonstrated lower levels of healthy microbiota (bifidobacteria, lactobacilli, propionibacteria, and eubacteria). In all the patients with CHF, propionibacteria counts were ≤50% versus the control group

Table 1. Clinical and anamnestic characteristics of groups

Parameter	Group 1, n=9	Group 2, n=18	Group 3, n=20	p		
				1 & 2	1 & 3	2 & 3
Age, years	63 (56; 74)	63.5 (61.3; 69)	61 (57.8; 65)	–	–	–
Body mass index, kg/m^2	30.8±3.9	31.3±5.5	28.3±4.3	–	–	0.048
Male/female, n	7/2	14/4	17/3	–	–	–
Chronic heart failure IIA/IIB/III, n	9/0/0	5/13/0	0/15/5	0.000	0.000	0.002
Decompensation/stable course, n	0/9	16/2	20/0	0.000	0.000	–
Atrial fibrillation, n	7	13	14	–	–	–
Chest X-ray (normal/stasis/ uni-/bilateral effusion), n	7/2/0/0	4/8/5/1	0/12/5/3	0.02	0.001	–
Ventricular heart rate, bpm	97±24	92±26	99±29	–	–	–
Systolic blood pressure, mm Hg	132±21	146±20	119±17	–	–	0.000
Diastolic blood pressure, mm Hg	82.1±4.4	83.9±2.9	72 (65; 80)	–	–	0.037
History of myocardial infarction, n (%)	3 (33.3)	9 (50)	10 (50)	–	–	–
Left ventricular ejection fraction, %	50.8 ±12.6	45.0±10.5	29.5 (25; 35)	–	0.006	0.001
Pulmonary artery systolic pressure, mm Hg	35.1±10.1	53.1±19.1	57.0 (50; 69)	0.035	0.000	–
Duration of chronic heart failure, months	60 (12; 84)	55 (10.5; 93)	18 (8; 40)	–	–	–
Deterioration, days	–	14 (14; 30)	25.5 (9.3; 60)	–	0.06	–
SHOKS score	5±2.2	11.8±4.0	13.4±3.6	0.001	0.000	–
6-minute Walk Distance, m	431±135	278±152	242±138	0.017	0.003	–

p, the level of significance of differences between Groups 1 and 2, Groups 1 and 3, and Groups 2 and 3, respectively.

(p<0.001). Bifidobacteria counts decreased with more severe CHF and were significantly different in Groups 2 and 3 versus the control value (p<0.001). In Group 1, bifidobacteria counts tended to decrease (p=0.077). Lactobacilli counts were similar in patients without CHF and those in Group 2, but these counts were significantly higher than those in Groups 1 and 3 (p=0.0002). Interestingly, Group 2 had higher absolute lactobacilli counts in the parietal layer than Groups 1 (p=0.004) and 3 (p=0.015). Patients with more severe CHF (Groups 2 and 3; p=0.072) showed maximum eubacteria counts as compared with Groups 1 and 2, with eubacteria counts being lower versus the control group (p=0.036 and 0.004, respectively). Blood levels of *Clostridium (C.) ramosum* marker were reduced significantly in patients with progressing CHF (Figure 1).

Patients with CHF had lower blood levels of fatty acids specific for *Ruminococcus, Streptococcus (Str.)* mutans, and *C.* difficile than those in the control group. Assays of clostridial and coccal intestinal bacteria in moderate CHF revealed associated increases in total coccal counts induced by higher levels of ruminococcus, staphylococcus, enterococcus, and anaerobic *Str. mutans* (Figure 2).

Group 2 had an increase in both total and individual actinomycetales counts. However, the estimated counts in the microbiocenosis of patients with CHF in all experimental groups were lower than the median values in the control group (p<0.01) (Figure 3).

A significant increase in the total microfungi counts was also identified in Group 2. However, these counts were lower than those in the control group (Figure 4). It should be noted that this trend was not relevant to all microfungi. For example, patients with CHF demonstrated higher aspergilla counts than the control group (p<0.05).

An increase in gram (-) bacteria (*Prevotella spp.* and *Kingella spp.*) counts were detected in Group 2 (Figure 5). In individual cases, other gram (-) bacteria (*Campylobacter mucosalis, Flavobacterium spp., Moraxella spp.*) were detected, more commonly in Groups 2 and 3.

The LPS levels naturally demonstrated a trend similar to gram (-) bacteria (0.20 [0.13; 0.25], 0.35 [0.30; 0.43] and 0.25 (0.18; 0.34) nmol/mL in Groups 1, 2 and 3, respectively). At the same time, the LPS levels were significantly higher in Group 2 versus Group 1 (p=0.042), and trended to be higher

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Table 2. Correlations of serum CRP and IL-6 and severity of CHF patient's condition

Parameter	C-reactive protein		Interleukin-6	
Stage of chronic heart failure	τ=0.35	p=0.016	τ=0.31	p=0.039
SHOKS score	τ=0.20	p=0.048	τ=0.22	p=0.151
Functional class	τ=0.21	p=0.037	τ=0.20	p=0.048
Severity of swelling	τ=0.38	p=0.01	τ=0.42	p=0.004
Severity of pulmonary congestion	τ=0.29	p=0.049	τ=0.22	p=0.144
Duration of worsening	τ=0.33	p=0.027	τ=0.10	p=0.527
NT-proBNP	τ=0.43	p=0.003	τ=0.55	p=0.0001

τ, the rank correlation coefficient, p, the level of significance; NT-proBNP, N-terminal pro-brain natriuretic peptide.

Table 3. Serum concentration of CSI factors in patients with CHF

Groups	NT-proBNP, pg/mL	CRP, mg/L	IL-6, pg/mL	IL-10, pg/mL
Group 1	109.0±21.0	4.89±1.22	2.72±0.83	1.8±0.70
Group 2	1328±113.0	9.20 (1.83; 11.5)	1.55 (0.83; 11.5)	1.10 (0.00; 5.33)
Group 3	3147 (2902; 3339)	12.10 (9.40; 12.7)	9.90 (5.33; 15.9)	2.90 (1.85; 5.75)
p (Groups 1 и 2)	0.000	>0.1	>0.1	>0.1
p (Groups 1 и 3)	0.000	0.001	0.002	0.05
p (Groups 2 и 3)	0.000	0.02	0.007	0.08

p (Groups 1 and 2), (Groups 1 and 3), (Groups 2 and 3), the level of significance of differences between Groups 1 and 2, Groups 1 and 3, and Groups 2 and 3, respectively; NT-proBNP, N-terminal pro-brain natriuretic peptide; CRP, C-reactive protein; IL-6, interleukin-6; IL-10, nterleukin-10.

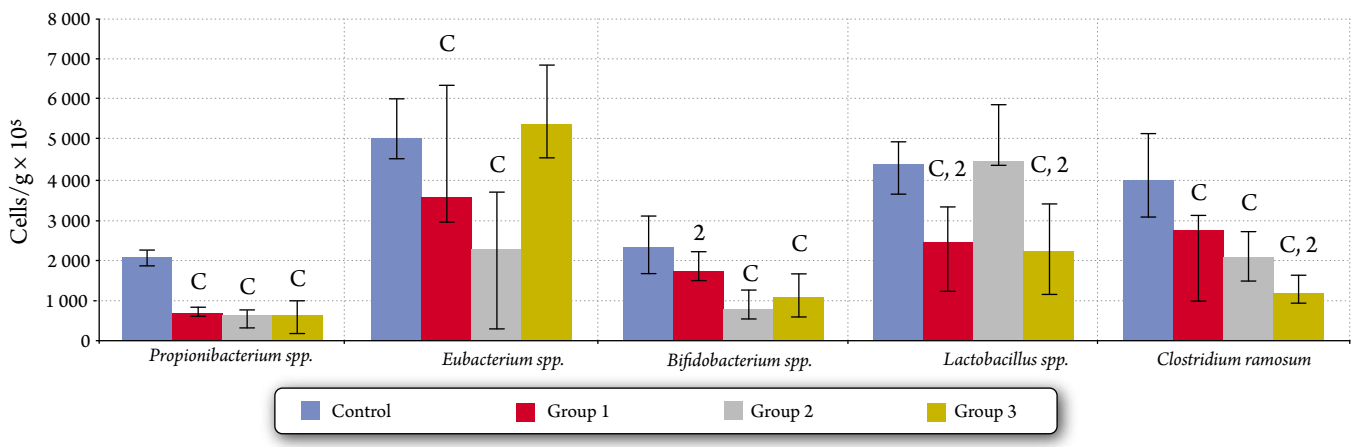
versus Group 3 (p=0.072). There were only five cases of LPS levels >0.5 nmol/mL (n=3 in Group 2, n=1 in Group 1 and n=1 Group 3). All CHF groups showed lower median LPS levels versus the control group (0.98 [0.47; 1.36] nmol/mL), p<0.05. Thus, we detected a comparable increase in microbial markers in Group 2 and minimal differences between Group 1 and Group 3.

Moderate direct correlations were found between *C. hystolyticum* (a common marker with *Str. pneumonia*), *Pseudonocardia spp., Aspergillus spp.* counts and the levels of IL-6 and IL-10 (Table 4).One-way reverse correlations were observed between the CK levels and *Propionibacterium* acnes and jensenii, *Streptomyces spp., Nocardia* asteroides counts. *Propionibacterium* acnes demonstrated negative correlations with all three studied inflammatory mediators. A negative correlation was found between IL-10 levels and *Staphylococcus aureus, C. difficile, C. ramosum, Eggerthella lenta,* and *Corynebacterium spp.* counts. There were positive correlations with *C. propionicum, Moraxella spp.* (a common marker with *Acinetobacter spp.*) and *Flavobacterium spp.* counts. It should be noted that the IL-6 levels were directly correlated with *Eubacterium spp.* counts and inversely correlated with *Ruminicoccus spp.* and *Streptomyces farmamarensis*. The latter showed a negative correlation with the CRP levels. No significant correlations between the CSI factors and total LPS levels were identified.

Discussion

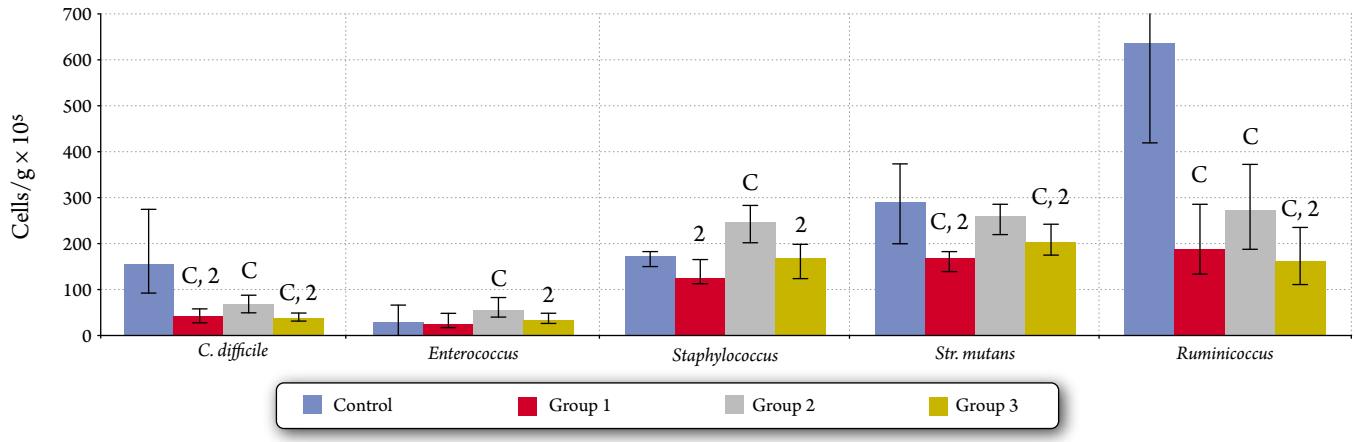
The reported CHF-associated CSI activation has been confirmed by the increased levels of key inflammatory

Figure 1. Parietal counts of normobiota in patients with CHF



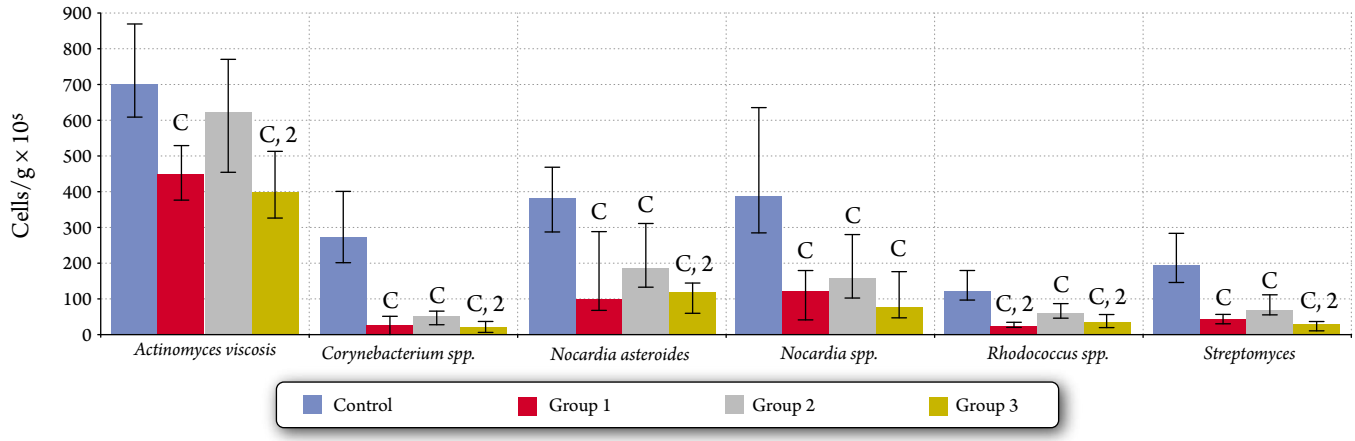
C — p<0.05 versus control, 2 — p<0.05 versus Group 2.

Figure 2. Parietal counts of clastridia and cocci in patients with CHN



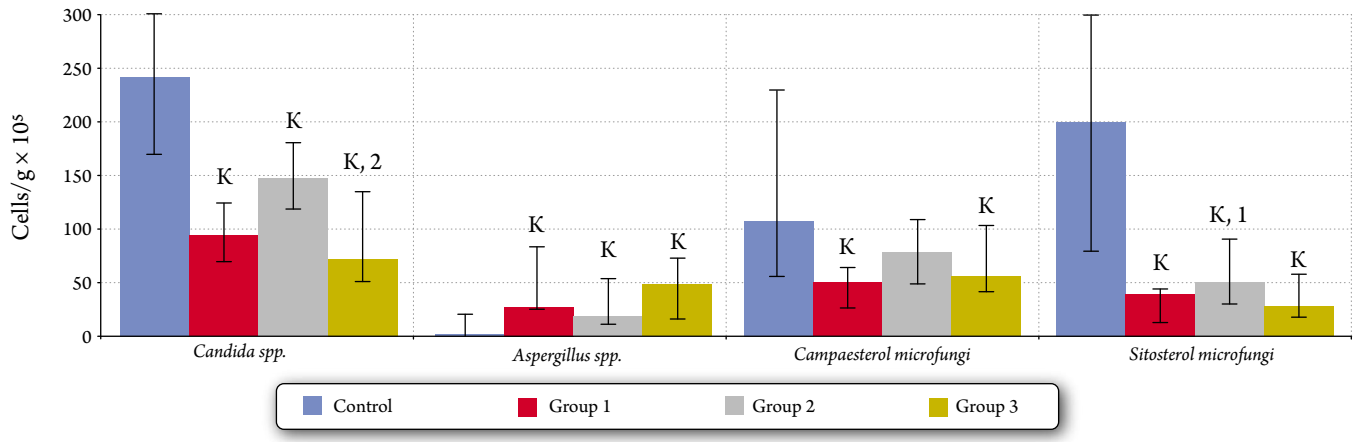
C — p<0.05 versus control, 2 — p<0.05 versus Group 2.

Figure 3. Parietal counts of actinomyces in patients with CHF



C — p<0.05 versus control, 2 — p<0.05 versus Group 2.

Figure 4. Parietal counts of microfungi in patients with CHF



C — p<0.05 versus control, 2 — p<0.05 versus Group 2.

mediators (CRP, IL-6, and IL-10) in a significant number of patients [4, 7, 14–16]. Potential mechanisms of CHF-associated hemodynamic and clinical effects of CKs can include adverse inotropic action, abnormal endothelium-dependent arteriolar dilatation, and effects on primary cellular and molecular genetic processes controlling cardiac remodeling, including hypertrophy, proliferation, necrosis, and cardiomyocyte apoptosis [1–3]. Positive correlations between the levels of NT-proBNP, IL-6, and IL-10 demonstrate an association of these CKs with mechanical extension of cardiac chambers and excessive activation of the renin-angiotensin-aldosterone system [3, 14]. The intensity and primary chronicity of immune

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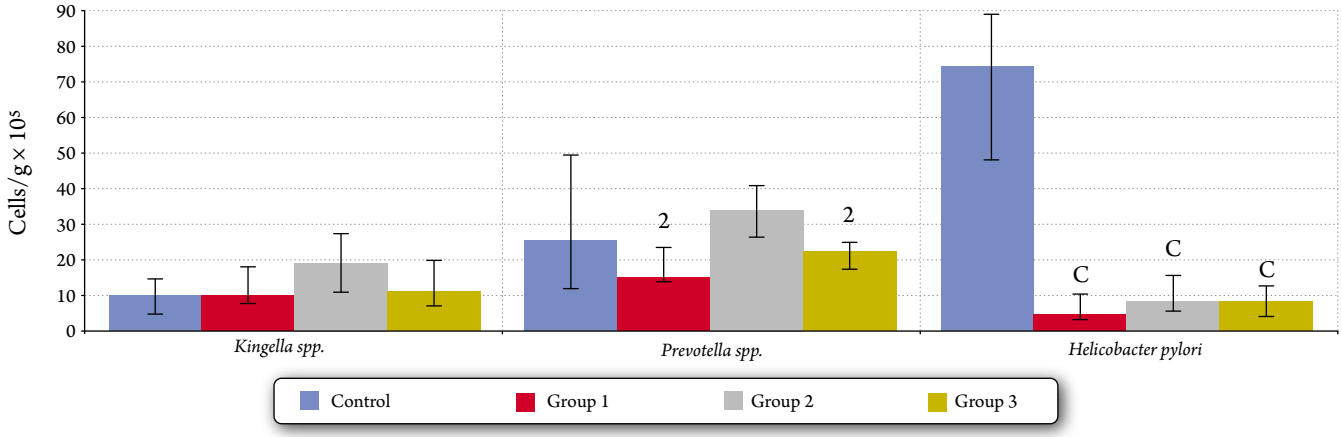
Figure 5. Parietal counts of gram(-) bacteria in patients with CHN



C — p<0.05 versus control, 2 — p<0.05 versus Group 2.

Table 4. Correlations of serum concentrations of CRP, IL-6, and IL-10 with the number of microorganisms in parietal microbiocenosis in patients with CHF

Microbiocenosis	CRP	IL-6	IL-10
Staphylococcus aureus	τ = 0,00, p=0,98	τ = –0,11, p=0,49	τ =–0,32, p=0,031
Clostridium difficile	τ = –0,15, p=0,31	τ = –0,26, p=0,08	τ = –0,44, p=0,0026
C. hystolyticum/Str. pneumonia	τ = 0,22, p=0,14	τ = 0,38, p=0,01	τ = 0,49, p=0,0007
C. propionicum	τ = –0,01, p=0,94	τ = 0,24, p=0,12	τ = 0,50, p=0,0005
C. ramosum	τ = 0,00, p=0,99	τ = –0,18, p=0,24	τ = –0,30, p=0,045
Eubacterium spp.	τ = 0,25, p=0,10	τ= 0,31, p=0,039	τ = 0,16, p=0,30
Eggerthella lenta	τ = 0,12, p=0,42	τ = 0,01, p=0,95	τ = –0,21, p=0,041
Propionibacterium acnes	τ = –0,32, p=0,028	τ = –0,52, p=0,0002	τ = –0,46, p=0,002
Propionibacterium jensenii	τ = –0,25, p=0,08	τ = –0,42, p=0,0045	τ = –0,49, p=0,0007
Ruminicoccus spp.	τ = –0,14, p=0,37	τ = –0,30, p=0,044	τ = –0,26, p=0,08
Corynebacterium spp.	τ = – 0,16, p=0,29	τ = – 0,28, p=0,06	τ = –0,48, p=0,0009
Nocardia asteroides	τ = –0,10, p=0,51	τ= – 0,30, p=0,043	τ = –0,40, p=0,006
Pseudonocardia spp.	τ = 0,02, p=0,96	τ= 0,36, p=0,014	τ = 0,51, p=0,0003
Streptomyces spp.	τ = –0,10, p=0,53	τ = –0,31, p=0,039	τ = –0,54, p=0,0000
Streptomyces farmamarensis	τ = –0,35, p=0,016	τ = –0,35, p=0,019	τ = –0,15, p=0,34
Flavobacterium spp.	τ = –0,19, p=0,21	τ = 0,00, p=1	τ = 0,34, p=0,023
Moraxella spp./Acinetobacter spp.	τ = 0,16, p=0,29	τ = 0,23, p=0,14	τ = 0,23, p=0,023
Aspergillus spp.	τ = 0,19, p=0,21	τ = 0,31, p=0,039	τ = 0,30, p=0,045

τ, the rank correlation coefficient, p, the level of significance; CRP, C-reactive protein; IL-6, interleukin-6; IL-10, interleukin-10.

responses are confirmed when CHF symptoms become more severe as in the case of a moderate increase in IL-10 levels, which despite being an anti-inflammatory CK, promotes the development of myocardial fibrosis [1].

Inflammatory mediators in patients with CHF are perceived to be produced by: cardiomyocytes and damaged intercellular matrix [14, 15], peripheral organ and tissue cells responding to catabolite accumulation in case of inadequate blood supply [15], and macrophages and other immune cells combating bacterial toxins penetrating through the edematous intestinal wall [4–9, 17]. Close, direct correlations of the levels of CRP and IL-6 with fluid retention syndrome and stage of

CHF reported here may characterize potential CSI activation mechanisms. This agrees with findings of Arutyunov et al. (2005) that inhibition of indigenous bacteria and proliferation of opportunistic bacteria in feces and parietal layer are associated with inflammatory activation in patients with CHF [7]. Egorova et al. (2012) demonstrated a direct correlation of the severity of dysbiosis, cytokinemia, and endotoxinemia with the severity of the CHF patient's condition [4]. Pasini et al. (2016) detected a direct correlation between the clinical severity, intestinal permeability, right atrial pressure, CRP levels, and the severity of dysbiosis [8]. The above papers mention that the activation of opportunistic bacteria was always associated with reduced intestinal commensal colonization (Bifidobacterium, Lactobacillus) [4, 7, 8]. Moreover, our findings showed a significant decrease in the counts of bifidobacteria, lactobacilli, and propionibacteria versus the control and reference values [12]. In the group of patients with NT-proBNP serum levels ≤2000 pg/mL, there was a direct correlation of changes in the counts of opportunistic bacteria and lactobacilli and a reverse correlation of changes in bifidobacteria counts with the severity of the patient's condition. In contrast, Cui et al. (2018) observed increased fecal counts of lactobacilli in patients with CHF, especially in an ischemic setting [18]. Kamo et al. (2017) examined fecal microbiota in 22 patients with symptomatic CHF and did not detect significant differences in the counts of bifidobacteria and lactobacilli versus those of healthy volunteers [11]. The authors found a significant decrease in relative eubacteria counts, as was found in this study. Most microbiocenosis parameters were similar in Groups 1 and 3. It seems that blood lipid homeostasis of microbial origin in patients with CHF cannot be described only by the quantitative and qualitative composition of intestinal microbiocenosis. It also depends on active and passive intestinal permeability for microbial markers [17]. Structural and functional alterations in intestinal walls are mainly caused by the severity of microcirculatory disorders, blood congestion, tissue swelling, collagen accumulation [4, 7], indigestion, food passage disorders, impaired synthesis of secretory immunoglobulins, and paretic stasis in immune lymphatic structures [19, 20].

It is suggested that severe decompensation associated with pronounced congestion, neurohumoral shifts, and activation of proinflammatory factors, trigger mechanisms inhibiting an increase in blood lipids of microbial origin. This could explain increased and decreased concentrations of most microbial markers in patients with moderate and severe decompensation of CHF, respectively. Given the above processes, we should point out a factor of fecal metabolomic potential in patients with CHF i.e., gene inhibition of active LPS transport proteins in the setting of activated synthesis of its components, which demonstrates the presence of microbiocenosis mechanisms preventing endotoxinemia [18]. Passive LPS diffusion through dystrophic intestinal mucosa should not be overrated in patients with CHF. Indeed, most papers did not report any significant increase of blood LPS above a conditional normal limit, although it correlated with the severity of CHF [4, 7, 8]. Given a role of microbiota in CSI progression, the almost linear increase in CK concentration with the severity of the patient's condition, and in the absence of a significant increase in the blood levels of most known markers of opportunistic pathogens, it appears that CHF-associated CSI intensity depends on local responsiveness of the intestinal immune system. This causes cytokinemia, prevention of massive microbial translocation to blood [17], and bacterial translocation to mesenteric lymph nodes [21]. This hypothesis and the significance of immunoinflammatory properties of opportunistic pathogens are confirmed by consistent correlations between their counts and levels of CSI mediators.

The direct correlation between LPS levels of *Flavobacterium spp.* and *Moraxella spp.* and the levels of IL-10 reported in our study can be associated with activated transcription of IL-10 genes [22], the role of which in the inhibition of LPS-mediated inflammation was also demonstrated by others [23]. Deficiency of normobiota and relevant short-chain fatty acids can promote LPS-induced inflammation by the effect on IL-10 production [24]. Lack of correlations between total LPS levels, Prevotella spp., Kingella spp., Helicobacter pylori marker levels, and CK levels can be associated with their immunological properties [25], selective barrier permeability, and body responsiveness.

A large variety of correlations identified between the counts of gram-positive bacteria and CK levels show their relevance in CSI activation. Thus, Bylova et al. confirmed that clostridia are involved in the development of CHF-associated CSI. Tuovinen et al. (2013) established that C. perfringens could stimulate the production of TNF-α and IL-10 by human mononuclear cells [27]. Elikowski et al. (2017) reported the development of Takotsubo cardiomyopathy associated with pseudomembranous colitis [28].

Several authors [29, 30] reported that individual actinomycetes and aspergilla could increase the levels of proinflammatory CKs. There are scarce reports in the literature on intestinal fungi, and these are limited to discussions of candida proliferation and differences of view on their role. Sterol-producing fungi identified by microbial marker mass-spectrometry may be involved in cholesterol metabolism. Sawamura et al. (2017) associate a decrease in blood levels of sterol with worse prognosis in patients with dilated cardiomyopathy; a point which warrants further study [31].

In our opinion, inverse correlations between CK levels and propionibacteria counts are expected since the latter are essential elements of metabolic integration of macro-, microorganisms, and advanced probiotics [32]. We detected an interesting correlation between streptomyces and nocardia

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counts and CRP levels that can be based on their ability to produce several antimicrobial substances and inhibit LPS-induced CK production of CKs by macrophages [33].

Conclusion

This study established that inflammatory mediators were significantly correlated with the severity of the condition in patients with CHF, with the severity of congestion and neurohumoral activation, and with intestinal microbiota counts. This indicates that stretching of the cardiac chambers, damage of peripheral cells, and the translocation of microbial components from the intestine in case of mucous congestion may be independent links of CSI pathogenesis and aggravate each other. The data suggest that therapeutic interventions could target the microbial-tissue complex to prevent the negative effects of CSI.

No conflict of interest is reported.

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