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THE EFFECT OF SHORT TERM TREATMENT WITH PROBIOTIC VSL#3 ON VARIOUS CLINICAL AND BIOCHEMICAL PARAMETERS IN PATIENTS WITH LIVER CIRRHOSIS

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The evidence is mounting that alterations of innate immunity and gut microbiota contribute to chronic liver disease and its complications. Modulation of intestinal microbiota is an emerging therapeutic strategy in hepatology. Probiotics through modulation of intestinal milieu have the potential to affect the course of liver disease. The data concerning the influence of probiotics on various plasma molecules and compounds involved in the pathogenesis of hyperdynamic circulatory state in liver cirrhosis is still not confluent and require further evaluation. In our study twenty patients with compensated and decompensated liver cirrhosis and ten healthy controls received probiotic VSL#3 daily for 28 days. Plasma levels of interleukin 6 (IL-6), vascular endothelial growth factor (VEGF), plasminogen activator inhibitor (PAI), macrophage inflammatory protein 3α (MIP-3 α/CCL20), monocyte chemotactic protein-1α (MCP-1/CCL2), human myeloperoxidase (MPO), nitric oxide (NO), prostaglandins, thromboxane (TXB_2) and big-endothelin were measured at baseline, day 14 and 28 of probiotic administration. The incidence of hepatic encephalopathy was assessed with critical flicker frequency. Changes in clinical, biochemical and microbiological parameters were evaluated. The stage of liver cirrhosis correlated with an increase in plasma levels of pro-inflammatory cytokines (IL-6) and chemotactic chemokines involved in immune cell trafficking (MIP-3α/CCL20). Probiotic administration in patients with liver cirrhosis led to modulation of plasma levels of several molecules and compounds measured (MIP-3α/CCL20, NO, big-endothelin, TXB_2 and MPO). The grade of encephalopathy during the course of probiotic supplementation remained unaffected in both groups of patients. VSL#3 treatment was well tolerated and safe in patients with liver disease. In patients with compensated and decompensated liver cirrhosis, VSL#3 manipulates selected plasma molecules and compounds involved in hyperdynamic circulatory dysfunction. Short term VSL#3 administration affects several clinical and biochemical parameters commonly altered in liver cirrhosis.

Key words: *probiotics, VSL#3, liver cirrhosis, portal hypertension, chemokines, macrophage inflammatory protein 3α, immune cell trafficking, endotoxin, microbiome, thromboxane B_2, myeloperoxidase*

INTRODUCTION

The evidence is mounting that gut microbiome alterations (dysbiosis) and intestinal mucosal injury are implicated in gut-liver-brain cross talk in liver disease (1, 2). The immunological status of the gut influences the structure and function of liver and brain (3) and could be viewed as a therapeutic target in hepatology. The number of plasma molecules and compounds implicated in the pathology of hyperdynamic circulatory state in liver cirrhosis is growing (4, 5). The increased activity of innate immunity system in the gut has been associated with elevated plasma levels of several molecules mediated by gut derived microbial products (4). The abnormal intestinal barrier function (6-8), microbiome alterations (9) and increased trafficking of pro inflammatory cells and molecules (10, 11) have been associated with more advanced intra-hepatic resistance (12) and bad prognosis in liver cirrhosis (13, 14).

Recently, the presence of the gut-vascular barrier (GVB) which controls the dissemination of bacteria into the bloodstream has been reported (8). GVB is composed of enteroglial cells and pericytes remaining in close contact with intestinal vascular endothelial cells and similar in structure to blood brain barrier (BBB). Both barriers are regulated by intestinal microbiota (8, 15). For example *Salmonella typhimurium*, Gram negative bacteria, by changing *wnt/beta catenin* signaling in the gut mucosa and endothelium was capable of disrupting the GVB, facilitating the spread of bacterial particles into portal and systemic circulation (8).

The evidence is mounting that systemic trafficking of monocytes and infiltration of peripheral tissues by gut-derived

macrophages (e.g. liver parenchyma and microglia) are regulated by intestinal microbiota (16, 17). Wild type CX3C chemokine receptor 1 deficient mice had higher number of infiltrating inflammatory cells (macrophages) in their livers and increased levels of TNF and MCP-1/CCL2 chemokines associated with impaired intestinal barrier function as compared to wild type control animals (18). Other study documented individual susceptibility to alcoholic liver disease driven by intestinal microbiota, associated with increased number of T lymphocyte subsets and natural killer T (NKT) lymphocytes in the liver, CD45+ lymphocytes in visceral adipose tissue and CD4+ and NKT in mesenteric lymph nodes in humanized germ-free and conventional animal models (19). Relevant to these examples, probiotic therapy was capable of altering gut-to-brain communication pathways and decreasing microglial activation and cerebral monocyte infiltration in animal models (20).

In humans several clinical studies aimed to target gut microbiome in order to improve hyperdynamic circulatory state - a hallmark of advanced cirrhosis (21). For example norfloxacin treatment partially reversed the hyperdynamic circulation in cirrhotic patients (22). Similarly rifaximin administration resulted in an improvement of systemic haemodynamics in patients with decompensated alcoholic cirrhosis with ascites (23) and encephalopathy (24). However data concerning the efficacy of probiotic administration in reducing the portal hypertension are conflicting. Several reports concerning probiotic use in patients with liver cirrhosis yielded promising results. Among beneficial effects observed were improvement of blood liver tests as well as reduction of plasma levels of endotoxin, aldosteron (25, 26), interleukin-1 (IL-1) and interleukin-6 (IL-6) (27) as well as an increase in serum sodium (28). Adjunctive probiotic (VSL#3) improved the response rate to propranolol therapy and was associated with decreases in TNF-α levels in peripheral and hepatic venous blood (29). Long-term use of VSL#3 administration has been associated with fewer hospital stays and better quality of life as well as decrease in liver disease clinical severity scores. Moreover there was a trend toward a reduction in the development of breakthrough hepatic episodes of encephalopathy among patients receiving the probiotic (27). Unfortunately studies investigating the effects of long term probiotic therapy report nearly 80% dropout rate of liver patients due to variety of clinical reasons (27).

Measuring the changes of plasma molecules in patients responding to probiotic therapy could lead to optimization of current therapeutic protocols and savings due to shifting patients from an in-patient to an out-patient care (30). It is particularly interesting to measure early response of plasma molecules to probiotic therapy (31) and correlate the findings with clinical status of chronic liver disease. For this purpose and based on available data derived from preclinical and clinical studies, we designed the prospective, non-randomized study to investigate the effect of 28 days administration of VSL#3 on variety of plasma molecules and compounds involved in the cascade of early events leading to altered hyperdynamic circulatory state in cirrhosis. We further thought to evaluate the effect of probiotic on the grade of hepatic encephalopathy and plasma biochemical profiles. Microbiological blood, stool and urine analysis served to monitor the safety and risk of infection of hospitalized patients receiving probiotics.

MATERIALS AND METHODS

Study protocol

This study was performed at Liver Unit, Pomeranian Medical University, a tertiary referral centre in Szczecin, Poland. All subjects signed the informed written consent before entering the study. Inclusion criteria were as follows: i) age between 18 and 75 years old ii) diagnosis of cirrhosis by means of liver biopsy or typical imaging studies. Patients presenting any of the following exclusion criteria were excluded from the study: i) active alcohol use (abstinence less than 6 months), ii) severe or clinically overt bacterial infection, administration of antibiotics, steroids, lactulose, lactitol or probiotics in last 3 months, iii) acute-on-chronic liver insufficiency, iv) hepatocellular carcinoma or other cancer, v) any other condition associated with immunodeficiency, vi) medication altering function of central nervous system (CNS), suffering from neurological or ophthalmological conditions, vii) active or past treatment with recombinant cytokines (e.g. anti-TNF, interferon etc.) viii) initiating the therapy with beta blockers within the prior 12 weeks, vi) pregnancy and vii) mental disease. All patients underwent upper gastrointestinal endoscopy prior to enrollment in order to identify signs of portal hypertension (e.g. esophageal or gastric varices). Protein intake was not restricted in any patient. All patients received standard hospital diet with average weight of 1.0 – 1.5 g/kg of daily protein. All patients were hospitalized at the time of study enrollment and led sedentary lifestyle with no or minimal exercising activity.

Ethics

The study protocol was approved by the Pomeranian Medical University ethics committee and conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008). This study was registered at ClinTrails.gov with the number NCT00831337.

Patients study

A group of 60 patients with liver cirrhosis were screened before enrollment. Thirty seven patients due to improper inclusion/exclusion criteria did not qualify for the study. Finally 23 consecutive patients diagnosed with compensated (CC) (n = 14) and decompensated (DC) liver cirrhosis with ascites (n = 9) as well as 10 age matched healthy controls (HC) were enrolled into the study. The Child-Pugh-Turcotte (CPT) liver disease clinical severity score served as determinant of severity of liver disease. Patients with CPT score 8 or less were classified as those with compensated cirrhosis (CPT grade A or B) and patients with CPT score 9 or more - classified as those with decopmensated liver cirrhosis (CPT grade C). One patient with compensated liver cirrhosis left the study because of a car accident and 2 patients with decompensated cirrhosis were excluded early in the course of the study either because of developing spontaneous bacterial peritonitis (SBP) or worsening of liver disease. Finally 13 patients with compensated and 7 patients with decompensated liver cirrhosis received probiotic treatment. VSL#3 is a probiotic mixture, frequently referred to in the literature, which contains eight, live lyophilized bacterial strains: *Bifidobacterium breve*, *Bifidobacterium longum* (*lactis*), *Bifidobacterium infantis* (*lactis*), *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Patients recruited and followed in the study had different etiologic factors behind their disease: alcohol (alc) in 11 patients; hepatatis B in 2 patients; hepatitis C in 4 patients; autoimmune hepatitis (AIH) in 1 patient, primary billiary cholangitis (PBC) in 1 patient; and 1 patient diagnosed with Wilson's disease.

Evaluation of levels of plasma molecules and compounds

In our study we aimed to measure variety of plasma molecules and compounds involved in the complex sequelae of events leading to hyperdynamic cirulatory state in cirrhosis. For this purpose the measured compounds were grouped based on their source of

synthesis and/or biological function: i) inflammatory: interleukin 6 (IL-6; HS600B R&D), plasminogen activator inhibitor (PAI-1; DSE100 R&D), human myeloperoxidase (MPO; DMY00R&D); iii) vasculogenic: vascular endothelial growth factor (VEGF; DVE00R&D), iv) vasodilatatory: nitric oxide (Total Nitric Oxide and Nitrate/Nitrite Parameter Assay KitKGE001R&D), prostaglandins (Prostaglandin $F_{2\alpha}$EIA Kit Cayman), v) vasoconstrictive: thromboxane B_2 (TXB_2; EIA Kit Cayman), Big Endotelin (Big ET; Biomedica Gruppe); vi) chemotactic - involved in cell trafficking: macrophage inflammatory protein 3 alpha (MIP-3α/CCL20; DM3A00R&D), monocyte chemotactic protein-1α (MCP-1/CCL2; DCP00R&D). All plasma compounds were measured using commercially available, high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol.

Critical flicker frequency and evaluation of hepatic encephalopathy

Critical flicker frequency (CFF) values were estimated using a portable, validated battery-powered analyser (HEPAtonorm Analyzer®; CyBio Robotics GmbH, Kusterdingen, Germany) as previously described (32). The analyser is designed to apply at the patient's bedside and it has been used by us in various other studies (33, 34). It consists of head-mounted goggles, a remote controller, a hand-held button and electrical leads for connecting the individual components. All data-acquisition and control functions are operated by the controller. *Via* a headset the controller evokes intrafoveal, red light pulses (wavelength 650 nm, luminance 270 cd/m^2, luminous intensity 5.3 mcd, 1:1 ratio between the visual impulse and the interval) with stepwise decreasing frequency from 60 Hz downward. This initial frequency of the light gives the patient the subjective impression of a steady light. The CFF threshold is determined when the impression of steady light changes to the flickering one. The patient has to register this change by pressing the button. After a brief instructions and training period the CFF values were measured 10 times and the mean values and standard deviation values from each patient were calculated. The whole procedure took about 20 min. All measurements were performed between 9 a.m. and 4 p.m. in a quiet, semi-darkening room after the initial dark adaptation phase before probiotic administration at day 0 and after 14 days of VSL#3 administration.

Microbiological and mycological analysis

Stool samples for bacteriological and mycological analysis were collected from all patients. Analysis was aimed to detect and count marker pathogenic bacteria. Material was collected from eight places following mixing of stool, to ensure homogeneity of a sample. Each sample was examined within 2 days of collection. Immediately after collection all samples were stored at 4°C and transported to the microbiological laboratory. Faeces were inoculated on a set of selective-differentiating and proliferative media. Growth of the following bacteria was analysed: *Escherichia coli* (including mucotic strains, lactose-negative), *Proteus*, *Pseudomonas*, other proteolytic bacteria (*Klebsiella*, *Citrobacter*, *Enterobacter*, *Serratia*), *Enterococcus*, *Bifidobacterium*, *Bacteroides*, *Lactobacillus* (including hydrogen peroxide strains) and bacteria of *Clostridium* genus. Total bacterial count was also determined. Microorganisms were inoculated and incubated in appropriate conditions, and were subject to further laboratory diagnostics. Faeces collected from patients was also analysed for occurrence and count of selected yeast-like fungi and moulds. Faeces samples were inoculated on Sabouraud agar medium and incubated for a definite time. Species identification was performed using chromogenous medium. Moulds were identified based on a direct preparation and a mycological key. Similarly blood and urine sample were collected and screened for the presence of pathogens at hospital laboratory in accordance with standard microbiological procedures.

Statistical analysis

The descriptive statistics were calculated as mean and standard deviation. To compare the mean values of the quantitative variables for the each examined plasma parameter (separately in three distinguished groups: healthy control (HC), compensated cirrhosis (CC) and decompensated cirrhosis (DC) at two time points, before probiotic administration and 28 days after, the t-test for dependent variables was used. Furthermore, the Kruskal-Wallis ANOVA with its post-hoc test was used to compare plasma parameters before probiotic administration between three groups: HC, CC and DC. In addition, the values of the examined plasma parameters were compared separately in three distinguished groups: HC, CC and DC at three time points, before probiotic administration, 14 days and 28 days after, the ANOVA Friedman test for dependent variables were used. In order to elaborate, both post-hoc tests for both the nonparametric Kruskal-Wallis ANOVA and the nonparametric Freidman ANOVA were based on the Dunn's method adapted to tied ranks which contains Bonfferoni correction for the multiple testing. All calculations were done in STATISTICA 12 software using all the tools available and closely following with their assumptions. The results were considered as statistical significance with $P < 0.05$.

RESULTS

General characteristics of patients with liver cirrhosis

General characteristics and medical history of patients diagnosed with liver cirrhosis and recruited into the study are listed in *Table 1*. All patients participants signed written consent and underwent clinical and laboratory evaluation before entering the study. There were 13 patients with CC with mean age 46 ± 27.7 years. Among them there were 5 females and 8 males. The group of DC constituted of 7 patients (2 females and 5 males) with the mean age of 42 ± 25.6 years. The most common etiologic factor of cirrhosis in both groups of patients was alcohol. All patients were administered diuretics and non-selective beta

Table 1. Demographic data of patients with liver cirrhosis.

Clinical data ^	**Liver cirrhosis (CC) compensated (n=13)**	**Liver cirrhosis (DC) decompensated (n=7)**
Age (mean ± S.D.)	46 ± 27.7	42 ± 25.6
Gender M / F	M – 8 / F – 5	M – 5 / F – 2
Aetiology		
Alcohol	6	5
Hepatitis B	1	1
Hepatitis C	3	1
AIH	1	
PBC	1	
Wilson's disease	1	
CPT Score	**A/B**	**C**
Medications		
Diuretics	all (13)	all (7)
Beta blockers	all (13)	all (7)

M, male; F, female; Alc, alcohol; AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; NS - non significant; S.D., standard deviation; CPT, Child Pugh Turcotte

Table 2. Laboratory data of patients with liver cirrhosis.

Laboratory data ^ (mean ± S.D.)	**Liver cirrhosis (CC) compensated**		**Liver cirrhosis (DC) decompensated**		**P**$^{*\#}$
	Day 0	Day 28	Day 0	Day 28	
ALT (U/L)	46 ± 27.7	42 ± 25.6	28 ± 22.2	39 ± 24.7	NS
AST (U/L)	46 ± 23.4	44 ± 21.8	67 ± 32.9	84 ± 24.7	NS
Creatinine (mg/dl)	0.7 ± 0.2	0.6 ± 0.1	0.8 ± 0.4	0.9 ± 0.3	NS
Albumin (g/l)	4.1 ± 0.3	4.1 ± 0.3	3.0 ± 0.3*	3.4 ± 0.6*	P < 0.05
INR	1.3 ± 0.2	1.2 ± 0.1	1.4 ± 0.2	1.3 ± 0.08	NS
CRP (mg/l)	5.3 ± 6.8	8.7 ± 15.8	15 ± 6.1	17 ± 9.2	NS
PLT (cells x 10^9)	188 ± 153	193 ± 157	124 ± 43.1	190 ± 96.2	NS
HGB (mmol/l)	12.6 ± 1.7	11.8 ± 1.7	9.5 ± 1.2*	12.9 ± 1.9*	P < 0.05
Sodium (mmol/l)	137 ± 2.2	135 ± 2.8	136 ± 3.5	136 ± 4.8	NS
MELD (mean ± S.D.)	9.8 ± 3.5	9.1 ± 2.2	14.4 ± 4.0*	11.7 ± 2.0*	P < 0.05

ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; PLT, platelets; HGB, hemoglobin; MELD, Model of End-Stage Liver Disease; NS - non significant, SD - standard deviation, *p-values relate to comparisons within the same groups before and after probiotic treatment (day 0 vs. day 28), the t-test for dependent variables was used. ^ Data of healthy controls are not shown - no statistical significance between the groups of patients with compensated cirrhosis and healthy controls, all biochemical results within normal range.

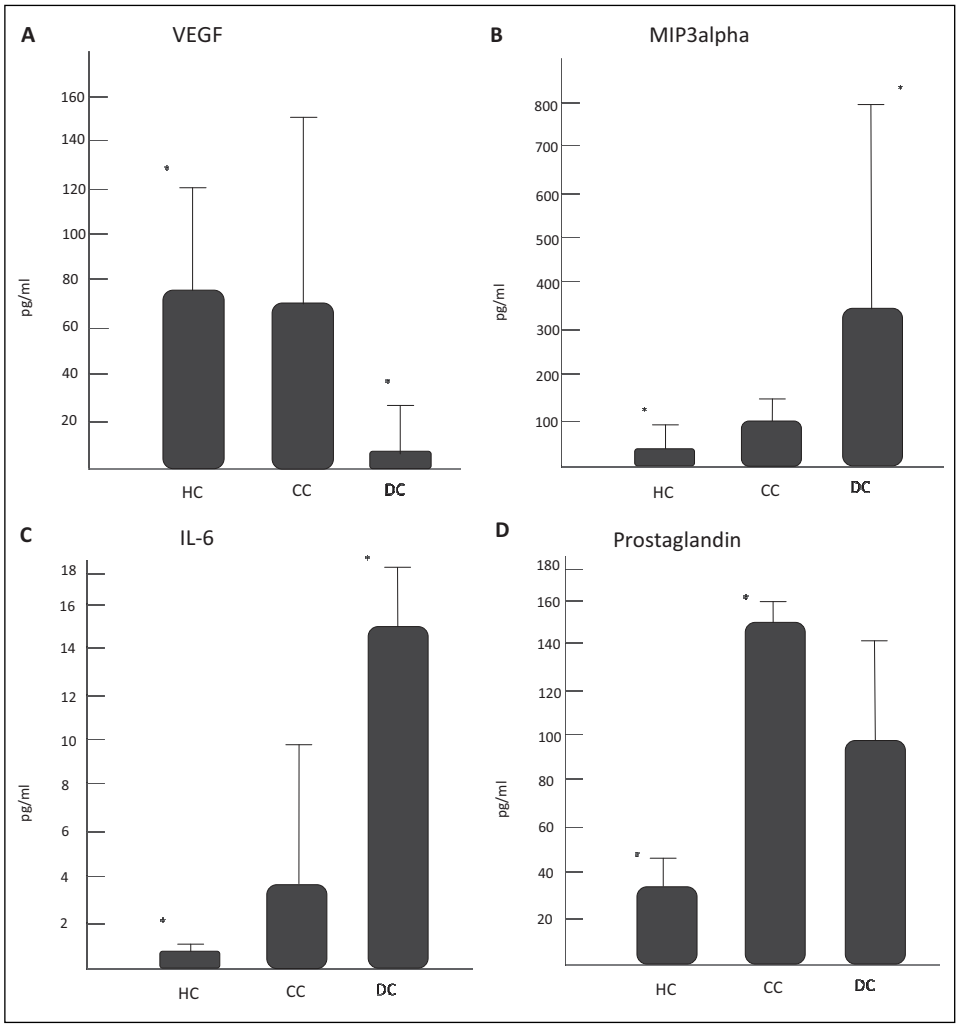

Fig. 1. The comparison of the values of plasma molecules at baseline - before probiotic administration - between three groups: healthy controls (HC), patients with compensated cirrhosis (CC) and decompensated cirrhosis (DC).

blockers with stable doses for more than 12 weeks before enrollment into the study. All biochemical results in patients with CC before and after probiotic administration were within normal range. Mean MELD score at the study entry in the group of patients with CC was 9.8 ± 3.5 and dropped down to 9.1 ± 2.2 after two weeks of probiotic supplementation. Patients with DC had abnormal levels of plasma

albumin (3.0 ± 0.3 g/dl) and hemoglobin (9.5 ± 1.2 g/dl) before the start of probiotic supplementation. Somehow surprising, in patients with DC at the end of probiotic administration the plasma levels of albumin and hemoglobin went up to 3.4 ± 0.6 g/dland 12.9 ± 2.9 g/dl accordingly ($P < 0.05$). At the beginning of the study, C-reactive protein (CRP) plasma levels were significantly higher in patients with DC in comparison to non-complicated liver disease (15.1 ± 6.8 g/dl vs. 5.3 ± 6.8 accordingly, $P < 0.005$). In CC patients probiotic treatment did not influence CRP and other biochemical parameters. MELD score in DC patients dropped down from 14.4 ± 4.0 before probiotic treatment to 11.7 ± 2.0 at the end of therapy ($P < 0.05$). The biochemical profiles in all HC were within normal range, did not differ statistically between the group of CC patients and were not manipulated by probiotic therapy (data not shown). The summary of demographic and biochemical data of patients with liver cirrhosis recruited into the study is presented in *Table 1* and *Table 2*.

Clinical and microbiological safety

In majority of patients VSL#3 administration led to an increase in total number of bacteria in the stool at the end of probiotic treatment ($P < 0.05$). None of the individuals enrolled into the study had positive microbiological stool, blood or urine samples tested for the presence of pathogenic bacteria during and after probiotic administration. Also none of the patients during VSL#3 supplementation required additional modification of the protocol requiring introduction of broad spectrum antibiotic. The probiotic therapy was well tolerated in all patients with liver disease. One healthy individual belonging to control

Table 3. The comparison of the parameter's values before probiotic administration between three groups: healthy controls (HC); patients with compensated cirrhosis (CC) and decompensated cirrhosis (DC). The $P < 0.05$ of the Kruskal-Wallis test shown the statistical significant difference between groups. The $P < 0.05$ of the post-hoc test shown the statistical significant difference between particular groups. * - statistical significance between groups (P-value of the post-hoc test); NO, nitric oxide; MPO, myeloperoxidase.

Parameter	Group	Mean ± S.D. (pg/ml)	P-value Kruskal-Wallis test	P-value post-hoc test
VEGF	HC*	76.94 ± 45.69	0.031	0.028*
	CC	67.74 ± 86.86		
	DC*	20.04 ± 18.10		
MCP1	HC	203.37 ± 82.22	0.157	
	CC	150.40 ± 64.52		
	DC	185.41 ± 22.47		
MIP3α	HC*	40.93 ± 47.24	0.014	0.013*
	CC	96.12 ± 58.87		
	DC*	350.60 ± 487.06		
Big endothelin	HC	0.67 ± 0.12	0.136	
	CC	0.93 ± 0.39		
	DC	0.81 ± 0.29		
Thromboxane	HC	231.21 ± 199.91	0.3	
	CC	853.64 ± 1351.09		
	DC	1337.88 ± 1886.01		
E1/PAI	HC	6.88 ± 4.32	0.072	
	CC	2.79 ± 0.78		
	DC	4.28 ± 2.85		
IL-6	HC*	0.70 ± 0.23	0.001	0.001*
	CC	4.00 ± 5.82		
	DC*	15.13 ± 4.35		
Prostaglandin	HC*	36.76 ± 10.36	0.003	0.003*
	CC*	155.87 ± 10.70		
	DC	99.51 ± 44.44		
NO	HC	20.50 ± 29.08	0.405	
	CC	22.81 ± 21.35		
	DC	46.75 ± 39.42		
MPO	HC	678.35 ± 175.18	0.373	
	CC	746.45 ± 339.43		
	DC	782.78 ± 138.66		

Table 4. Plasma concentrations of molecules manipulated by VSL#3 administration in patients with decompensated (DC) and compensated (CC) liver cirrhosis and healthy controls (HC) at different time points (day 0, 14 and 28). Asterisk indicates a significant change at different time points ($p < 0.05$).

VSL#3	Patients	Day 0 (pg/ml)	Day 14 (pg/ml)	Day 28 (pg/ml)	P value
Chemoatractive chemokines					
MCP1/CCL2	HC	203.37 ± 82.22	204.25 ± 55.77*	162.79 ± 34.23*	0.049
	CC	150.39 ± 64.52	168.45 ± 73.28	140.58 ± 62.76	0.12
	DC	185.40 ± 22.46	266.51 ± 98.08	170.12 ± 154.55	0.16
MIP3α/CCL20	HC	40.92 ± 47.23	67.23 ± 58.36*	19.87 ± 9.74*	0.028
	CC	96.12 ± 58.87*	105.49 ± 80.88	63.8 ± 36.87*	0.016
	DC	400.99 ± 547.15	334.55 ± 362.03	101 ± 0.0 ^	0.10 ^
Vasoactive molecules and compounds					
Big endotelin	HC	0.67 ± 0.11	0.84 ± 0.25	0.55 ± 0.15	0.10
	CC	0.93 ± 0.39	0.95 ± 0.41	34.42 ± 49.93	0.013
	DC	0.81 ± 0.28*	0.83 ± 0.20	80.97 ± 44.77*	0.04
TXB_2	HC	231.20 ± 199.90	368.99 ± 281.00	180.32 ± 114.09	0.65
	CC	853.64 ± 1351.09*	1954.89 ± 1820.04*	113.15 ± 63.58*	0.003
	DC	1336.87 ± 1816.01*	1506.62 ± 1853.73	169.15 ± 152.39*	0.002
Prostaglandin	HC	36.75 ± 10.35*	101.47 ± 151.47*	1.13 ± 0.36*	0.00506
	CC	155.87 ± 110.70	200.30 ± 263.89	79.32 ± 34.85	0.23
	DC	99.51 ± 44.44	183.25 ± 177.53	103.23 ± 4.99	0.81
NO	HC	20.50 ± 29.08	13.35 ± 9.84	18.97 ± 15.78	0.86
	CC	22.81 ± 21.34	23.68 ± 36.72	53.42 ± 44.35	0.12
	DC	46.75 ± 39.42	63.89 ± 14.95*	107.89 ± 15.41*	0.022
MPO	HC	678.34 ± 175.17	978.35 ± 1016.22	587.71 ± 94.64	0.65
	CC	746.44 ± 339.43*	562.95 ± 195.59	470.57 ± 331.47*	0.031
	DC	782.77 ± 138.65	2745.23 ± 3791.40*	191.44 ± 202.22*	0.022

Table 5. Plasma concentrations of molecules not affected by VSL#3 treatment in all groups studied at different time points (day 0, 14 and 28).

VSL#3	Patients	Day 0 (pg/ml)	Day 14 (pg/ml)	Day 28 (pg/ml)	P value
Vasculogenic chemokines					
VEGF	HC	76.94 ±45.68	71.43 ± 27.73	63.58 ± 17.29	0.65
	CC	67.73 ± 86.85	69.58 ± 73.03	64.00 ± 38.48	0.54
	DC	20.03 ± 18.10	46.38 ± 47.44	88.30 ± 28.38	0.16
Proinflammatory molecules					
E1/PAI	HC	6.87 ± 4.31	7.31 ± 2.59	4.86 ± 3.13	0.36
	CC	2.78 ± 0.78	4.42 ± 2.71	46.29 ± 51.90	0.71
	DC	4.27 ± 2.85	5.06 ± 3.53	61.87 ± 53.58	0.44
IL-6	HC	0.70 ± 0.22*	1.14 ± 0.66	3.29 ± 3.46*	0.005#
	CC	3.99 ± 5.82	4.54 ± 5.76	46 ± 52.19	0.45
	DC	14.19 ± 4.40	13.67 ± 5.16	101.0000	0.04

The data presented as Mean ± standard deviation. The results of ANOVA Friedman analysis (Friedman repeated measures analysis of variance by ranks) with Kendall's coefficient of concordance with post hoc test. Analysis of changes of serum levels of plasma molecules (independently for each molecule) in groups of patients studied (healthy controls. compensated and decompensated cirrhosis) in day 0, 14 and 28. Asterisk indicates a significant change at different time points ($p < 0.05$). Hash # means a change of value which is clinically irrelevant. HC, healthy controls; CC, compensated cirrhosis; DC, decompensated cirrhosis; VEGF, vascular endothelial growth factor; PAI, plasminogen activator inhibitor; IL-6, interleukin-6.

group developed short lasting and mild episode of diarrhoea associated with flatulence and abdominal cramps at the beginning of probiotic intake, however the symptoms resolved shortly with the continuation of therapy.

Plasma concentration of molecules and compounds involved in the pathogenesis of hyperdynamic circulatory state in patients with liver cirrhosis

Different plasma levels of molecules and compounds were measured in plasma of patients with liver cirrhosis. DC patients manifested the highest levels of pro-inflammatory cytokines, with IL-6 most significantly increased (P = 0.009) (14.19 ± 4.4 pg/ml) at the study baseline.

In comparison Il-6 plasma levels were much lower in CC patients (3.99 ± 5.82 pg/ml) or HC (0.70 ± 0.22 pg/ml). DC patients presented also with significantly higher plasma levels of chemotactic chemokine MIP-3α/CCL20 in comparison to CC patients and healthy individuals. Of intriguing interest were the plasma levels chemokine MIP-3α/CCL20, which were almost 10-fold higher (350.60 ± 487.06 pg/ml) in DC patients than in HC (40.93 ± 47.24 pg/ml) (P-value, post-hoc test P = 0.013). Contrary to these observations patients with DC presented with the lowest plasma levels of vasculogenic cytokine VEGF (20.04 ± 18.10 pg/ml) in comparison to HC (76.94 ± 45.69 pg/ml) (P-value, post-hoc test P = 0.028). Additionally plasma levels of potent vasodilatators prostaglandins differed between CC patients (155.87 ± 10.70) and HC (36.76 ± 10.36) (P-value, post-

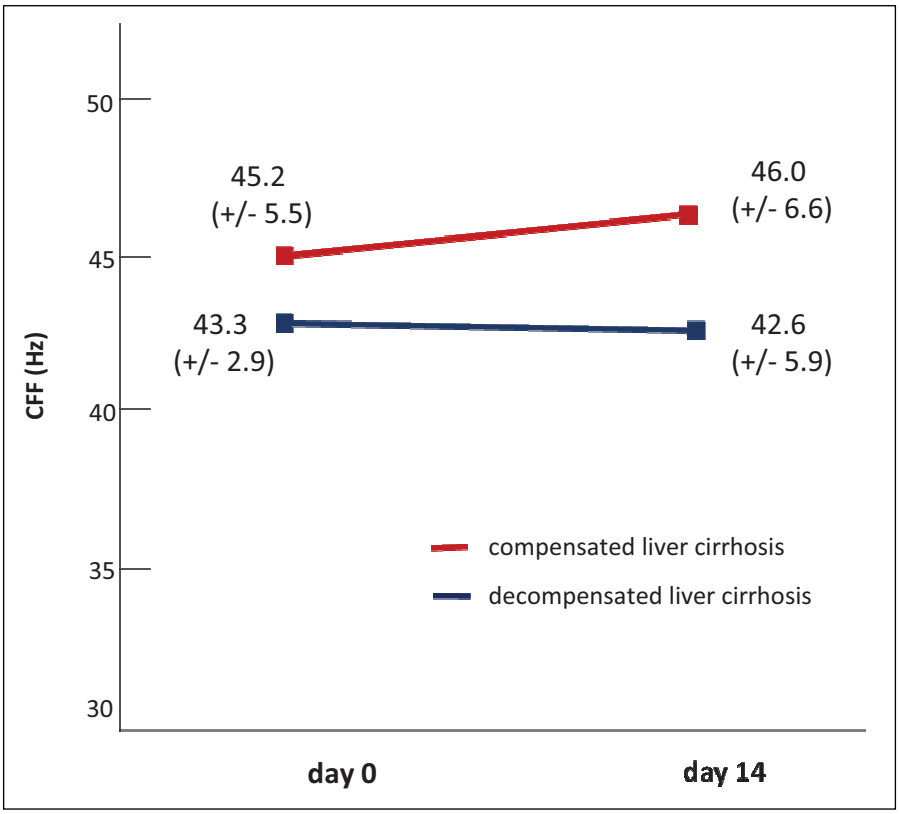

Fig. 2. Evaluation of encephalopathy using critical flicker frequency in patients with compensated and decompensated liver cirrhosis before and after probiotic administration (day 0 and 14).

hoc test P = 0.03). The baseline levels of all plasma molecules in all groups of patients before administration of probiotic (day 0) are presented in *Table 3* and *Fig. 1*.

The effect of VSL#3 administration on the levels of plasma molecules in patients with decompensated liver cirrhosis

The levels of several molecules and compound in plasma of DC patients, were manipulated at the end of VSL#3 supplementation: vasodilatatory NO and vasoconstrictive big-endothelin and TXB_2. Other molecules tested were not influenced by probiotic administration and did not change significantly over 28 days of therapy. To our surprise NO and big-endothelin plasma levels in DC patients were significantly increased at the end of probiotic administration and differed significantly in comparison to the levels found in CC and HC. In contrast plasma TXB_2 levels decreased as measured at day 28 of therapy (1336 ± 1816 pg/ml versus 169.15 ± 152.39 pg.ml, P < 0.002). Moreover we observed a tendency to an increase in VEGF expression in some patients with DC at the end of the treatment but these results did not reach statistical significance (20.03 ± 18.10 versus 46.38 ± 47.44 respectively, P = 0.16).

The compliance among DC patients was good and VSL#3 was administered for full 28 days. However data concerning evaluation of MIP-3α/CCL20 levels in DC patients after 28 days of probiotic administration in our study are inconclusive due to possible sample processing error.

VSL#3 administration modulate the levels of plasma molecules in patients with compensated liver cirrhosis and healthy individuals

In patients with compensated cirrhosis (CPT < 8), we were able to observe changes in the levels of several plasma molecules on day 28 of probiotic therapy. We observed differences in the plasma levels of chemotactic chemokine CCL20/MIP-3α between day 0 (96.12 ± 58.87 pg/ml) and day 28 (63.8 ± 36.87 pg/ml), P = 0.01; vasoconstrictive big-endothelin - day 0 (0.93 ± 0.39 pg/ml) and day 28 (34.42 ± 49.93 pg/ml), P = 0.1; and TXB_2 - day 0 (853.64 ± 1351.09) and day 28 (113.15 ± 63.58), P = 0.003. MPO plasma levels changed from day 0 (746.44 ± 339.43) to day 28 (470.57 ± 331.47), P = 0.03. Of note none of the plasma molecules were manipulated on day 14 of probiotic supplementation. Other molecules tested were not affected by probiotic treatment. Of interest probiotic therapy led to changes of plasma molecule also in healthy individuals. Among tested molecules the plasma levels of MCP-1/CCL2, (P = 0.04) and prostaglandins (P = 0.005) were manipulated by probiotic on day 28 of therapy. The summaries of plasma molecules profiles in all groups studied are presented in *Table 4*.

Evaluation of hepatic encephalopathy

There was no significant difference between mean CFF values when patients with compensated and decompensated liver cirrhosis were analyzed (45.9 Hz ± 5.4 versus 43.3 Hz ± 2.9). CFF values were not affected by probiotic administration in all patients examined (45.9 Hz ± 5.4 at day 0 versus 46.6 Hz ± 6.7 at day 14 in compensated cirrhosis and 43.3 ± 2.9 at day 0 versus 42.6 ± 5.4 at day 14 in decompensated cirrhosis). CFF values did not differ between groups after patients stratification to gender, level of education or etiology of the liver disease.

The mean values of experiments evaluating grade of hepatic encephalopathy before and after VSL#3 probiotic supplementation are presented in *Fig. 2*.

DISCUSSION

Portal hypertension is a clinical syndrome associated with liver cirrhosis and responsible for the onset of serious life threatening complications (35). The current hypothesis behind development and progression of liver disease revolves around

microbiome, innate immunity and intestinal barrier alterations. Structural (liver, blood vessels) and functional (blood, lymph) abnormalities involve complex interplay between variety of pro-inflammatory and vasoactive molecules very often exerting pleiotropic biological impact. Probiotics are capable of modulating gut microbiota and have been shown to exert positive effects on various biochemical parameters associated with portal hypertension in patients with liver cirrhosis.

For example Tandon *et al.* (26) in a small pilot study of patients with compensated cirrhosis, observed the reduction of endotoxin and plasma levels of aldosterone after VSL#3 administration. In the study of Jayakumar *et al.* (25) probiotic administration was associated with a small reduction in aldosterone but without the effect on the severity of portal hypertension as assessed by measuring hepatic venous pressure gradient (HVPG). In contrast to this observation Rincon *et al.* (28) observed improved hepatic and systemic haemodynamics in patients with decompensated cirrhosis after supplementation with probiotic VSL#3. These changes were associated with improvement in the levels of serum sodium. Dhiman *et al.* (27) in a study of six month duration were able to document decrease in plasma levels of TNF-α, IL1β, IL-6 as well as levels of plasma renin and brain natriuretic peptide after 24 days of VSL#3 supplementation. After six month of VSL#3 therapy Dhiman *et al.* (27) also reported fewer hospital stays, improved quality of life and decrease in CTP and Model for End-Stage Liver Disease (MELD) scores among patients with advanced liver cirrhosis. The authors observed the trend toward a reduction in the development of hepatic encephalopathy episodes among patients receiving VSL#3 probiotic but reported nearly 80% dropout rate during duration of the trial (27).

In our study we become interested to evaluate the levels of various plasma molecules which could be involved in the early stages of the development of hyper dynamic circulatory state in liver cirrhosis. In our non-randomized prospective study we were particularly interested in measuring plasma molecules with pro-inflammatory and vaso-active biologic functions. With no surprise the highest levels of pro-inflammatory cytokines were observed in plasma of DC patients (CPT > 8). Levels of IL-6 were significantly increased in comparison to values found in patients with compensated disease (CPT < 8). Plasma molecules tested were manipulated by VSL#3 in the group of patients with DC at day 28 of study duration. At the end of probiotic administration we observed significant increase in plasma levels of NO and big-endothelin as well as decrease of TXB_2 and MPO in DC patients. This observation is somehow surprising as endothelins are well known vasoconstrictor peptides (36) and plasma levels of these molecules correlated with the severity of liver disease and portal pressure in previous studies (37). However Vaughan *et al.* observed that infusion of endothelin-1 into the forearm of patients with advanced liver disease elicited a vasodilatatory effect. Contrasting vasoconstrictive effect was observed after infusion of endothelin-1 into the forearm of healthy individuals (38). Wereszczynska-Siemiatowska *et al.* documented significant increase in peripheral endothelin-1 levels in patients with liver cirrhosis and non-bleeding esophageal varices receiving non-selective beta-blocker therapy for more than 4 months (39). In our study we observed decrease in plasma levels of another compound TXB_2 (vasoconstrictor) and increase in plasma levels of NO (vasodilatator). It might be tempting to speculate, that the observed in our study changes in plasma levels of vasoactive compounds could have additive and synergistic effect on splachnic circulation evoked by VSL#3 supplementation. Of note other paracrine and autocrine mechanisms, resulting from manipulation by VSL3#, such as stimulation of cellular growth, cell trafficking and proliferation affecting hepatic and vascular tissue components might be involved (40).

In our study baseline plasma levels of interleukin-6 and chemoatractant chemokine MIP-3α/CCL20 correlated with disease severity. The highest plasma levels of these molecules were present in DC patients. The exact mechanism determining the effect of probiotics on hyperdynamic circulatory state in liver cirrhosis remains not well understood. Gut mediated production of pro-inflammatory plasma molecules contribute to progression of portal hypertension. These changes occur early in the sequence of the pathogenic cascade of events leading to renal failure, spontaneous bacterial peritonitis (SBP) and development of HRS (41). In our trial VSL# administration had no effect on the plasma levels of IL-6 in patients with liver cirrhosis. In similar study Rincon *et al.* (28) documented the reduction of portal pressure after VSL#3 administration, but reported that changes in portal pressure were unrelated to systemic inflammation, bacterial translocation or NO levels. The authors suggested the possible involvement of other, yet undisclosed factors contributing to observed phenomena. Relevant to this report is our observation that VSL#3 administration was associated with changes in plasma levels of CCL-2 cytokine in healthy individuals and MIP-3α/CCL20 in controls and patients with CCL2 and CCL20 chemokines recruit tissue-dependent infiltrating monocytes and macrophages involved in inflammation-associated angiogenesis involved in worsening of liver fibrosis (42). MIP-3α/CCL20 chemokine mediates it's deleterious effect on the liver through bacterial LPS and has recently been proposed to be a marker of hepatic inflammation, injury and fibrosis in patients with alcoholic hepatitis (4). For example Kawano *et al.* were able to prevent HFD-induced insulin resistance in animal model by inducing the inhibition of pro-inflammatory colonic-derived macrophage infiltration in peripheral tissue (43).

In patients with compensated disease we were also able to observe positive effect of VSL#3 on plasma profiles of other molecules such as TXB_2 and MPO. These changes were noticeable only after day 28 of therapy. On day 14 none of the molecules were manipulated by VSL#3.

As the progression of liver cirrhosis might be mediated by changes in microcirculatory milieu of intestinal mucosa (14), frequently disturbed in liver cirrhosis (44, 45) we become interested to measure plasma levels of VEGF and other molecules involved in vasculogenesis and maintenance of mucosal and vascular gut barriers. Recently, an acute phase protein-plasminogen activator inhibitor-1 (PAI-1) has been associated with the increased risk of cardiovascular disease, obesity, insulin resistance and inflammation. Plasma levels of PAI-1 correlated with progression of hepatic steatosis (46). These positive correlations were independent of body mass index, visceral fat, insulin resistance, and inflammatory markers. However, in our study PAI-1 plasma levels were not different between groups at the beginning and end of probiotic supplementation.

In addition to evaluating the effect of VSL#3 on plasma profiles of various molecules we tempted to measure grade of hepatic encephalopathy and its responsiveness to probiotic administration. In our study the grade of encephalopathy was similar in both groups of patients with liver disease (CC versus DC) and again not manipulated by probiotic supplementation. Despite positive outcomes of probiotics on grade of hepatic encephalopathy in smaller studies (47), larger systemic analysis yielded less optimistic results. One of the Cochrane's studies comparing the effect of probiotics to no treatment in 550 patients with hepatic encephalopathy enrolled into 7 clinical trials ended with following conclusions: no significant difference in all-cause mortality, recovery from hepatic encephalopathy, adverse events,

quality of life, or need for modulation of treatment, despite noticed reduction in plasma ammonia levels in cirrhotic patients (48). More recent, well-designed, prospective randomized trial revealed that primary endpoint, which was the rate of recurrent/breakthrough OHE, was not statistically lower on probiotics (28).

In our observation the probiotic therapy was well tolerated and safe. None of the patients developed serious infection, which would require administration of broad spectrum antibiotic or other rescue medication. VSL#3 treatment was well tolerated in all patients with liver cirrhosis ($P < 0.05$). None of the individuals complained of any discomfort or abdominal distress during the treatment. All blood, stool and urine microbiological tests were negative for the presence of pathogens. Finally VSL#3 administration led to reduction in MELD score of DC patients at day 28. We are aware that overall number of patients recruited into our study is too small to draw safe conclusions. However our enrollment took place only among patients being hospitalized. This group represents special group of patients with liver cirrhosis highly endangered with complications and need for rescue therapy. Study enrollment and compliance in other projects yielded similar problems (25-28).

Concluding the results from our and other studies the duration of probiotic treatment in patients with liver cirrhosis seems to be of critical importance (28, 49). Long term probiotic administration in patients with advanced liver cirrhosis exert a challenge and might be interrupted by variety of clinical reasons (28, 50). The action of probiotics is modest (51), however in patients with compensated and decompensated liver cirrhosis, we were able to detect changes of plasma molecules after VSL#3 administration. The grade of encephalopathy during the course of probiotic supplementation remained unaffected in all groups of patients. Of clinical importance VSL#3 treatment was well tolerated, safe and led to improvement of several clinical and biochemical parameters in patients with liver disease.

Based on growing body of experimental and clinical evidence supporting the hypothesis that the intestinal microbiota influences the gut immunological status and function of the liver (52-55) it is worth investigating the role of different probiotic formulations in large prospective and randomized trials. Of clinical importance are also other investigations relating to other compounds with potential to affect the immunological status of gut and liver axis (59-65).

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REFERENCES

1. Haque TR, Barritt AS. Intestinal microbiota in liver disease. *Best Pract Res Clin Gastroenterol* 2016; 30: 133-142.
2. Ge PS, Runyon BA. Treatment of patients with cirrhosis. *N Engl J Med* 2016; 375: 767-777.
3. Ahluwalia V, Betrapally NS, Hylemon PB, *et al.* Impaired gut-liver-brain axis in patients with cirrhosis. *Sci Rep* 2016; 6: 26800. doi: 10.1038/srep26800.
4. Affo S, Morales-Ibanez O, Rodrigo-Torres D, *et al.* CCL20 mediates lipopolysaccharide induced liver injury and is a potential driver of inflammation and fibrosis in alcoholic hepatitis. *Gut* 2014; 63: 1782-1792.
5. Koyama Y, Xu J, Liu X, Brenner DA. New developments on the treatment of liver fibrosis. *Dig Dis* 2016; 34: 589-596.
6. Kalaitzakis E, Johansson JE, Bjarnason I, Bjornsson E. Intestinal permeability in cirrhotic patients with and without ascites. *Scand J Gastroenterol* 2006; 41: 326-330.
7. Scarpellini E, Valenza V, Gabrielli M, *et al.* Intestinal permeability in cirrhotic patients with and without spontaneous bacterial peritonitis: is the ring closed? *Am J Gastroenterol* 2010; 105: 323-327.
8. Spadoni I, Zagato E, Bertocchi A, *et al.* A gut-vascular barrier controls the systemic dissemination of bacteria. *Science* 2015; 350: 830-834.
9. Bajaj JS, Heuman DM, Hylemon PB, *et al.* Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014; 60: 940-947.
10. Du Plessis J, Vanheel H, Janssen CE, *et al.* Activated intestinal macrophages in patients with cirrhosis release NO and IL-6 that may disrupt intestinal barrier function. *J Hepatol* 2013; 58: 1125-1132.
11. Handa P, Kowdley KV. Chemokines: potent mediators of hepatic inflammation and fibrosis in chronic liver diseases. *Ann Hepatol* 2013-2014; 13: 152-154.
12. Claria J, Stauber RE, Coenraad MJ, *et al.* Systemic inflammation in decompensated cirrhosis: Characterization and role in acute-on-chronic liver failure. *Hepatology* 2016; 64: 1249- 1264.
13. Purohit V, Bode JC, Bode C, *et al.* Alcohol, intestinal bacterial growth, intestinal permeability to endotoxin, and medical consequences: summary of a symposium. *Alcohol* 2008; 42: 349-361.
14. Rosselli M, MacNaughtan J, Jalan R, Pinzani M. Beyond scoring: a modern interpretation of disease progression in chronic liver disease. *Gut* 2013; 62: 1234-1241.
15. Michel L, Prat A. One more role for the gut: microbiota and blood brain barrier. *Ann Transl Med* 2016; 4: 15. doi: 10.3978/j.issn.2305-5839.2015.10.16
16. Natasi C, Candela M, Bonefeld CM, *et al.* The effect of short-chain fatty acids on human monocyte-derived dendritic cells. *Sci Rep* 2015; 5: 16148. doi: 10.1038/srep16148
17. Benakis C, Brea D, Caballero S, *et al.* Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. *Nat Med* 2016; 22: 516-23.
18. Schneider KM, Bieghs V, Heymann F, *et al.* CX3CR1 is a gatekeeper for intestinal barrier integrity in mice: limiting steatohepatitis by maintaining intestinal homeostasis. *Hepatology* 2015; 62: 1405-1416.
19. Llopis M, Cassard AM, Wrzosek L, *et al.* Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. *Gut* 2016; 65: 830-839.
20. D'Mello C, Ronaghan N, Zaheer R, *et al.* Probiotics improve inflammation-associated sickness behavior by altering communication between the peripheral immune system and the brain. *J Neurosci* 2015; 35: 10821-10830.
21. Moller S, Hobolth L, Winkler C, Bendtsen F, Christensen E. Determinants of the hyperdynamic circulation and central hypovolaemia in cirrhosis. *Gut* 2011; 60:1254-1259.
22. Rasaratnam B, Kaye D, Jennings G, Dudley F, Chin-Dusting J. The effect of selective intestinal decontamination on the hyperdynamic circulatory state in cirrhosis. A randomized trial. *Ann Intern Med* 2003; 139: 186-193.
23. Kalambokis GN, Mouzaki A, Rodi M, *et al.* Rifaximin improves systemic hemodynamics and renal function in patients with alcohol-related cirrhosis and ascites. *Clin Gastroenterol Hepatol* 2012; 10: 815-818.
24. Orr JG, Currie CJ, Berni E, *et al.* The impact on hospital resource utilisation of treatment of hepatic encephalopathy with rifaximin-α. *Liver Int* 2016; 36: 1295-1303.

25. Jayakumar S, Carbonneau M, Hotte N, *et al.* VSL#3 probiotic therapy does not reduce portal pressures in patients with decompensated cirrhosis. *Liver Int* 2013; 33: 1470-1477.
26. Tandon P, Moncrief K, Madsen K, *et al.* Effects of probiotic therapy on portal pressure in patients with cirrhosis: a pilot study. *Liver Int* 2009; 29: 1110-1115.
27. Dhiman RK, Rana B, Agrawal S, *et al.* Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: a randomized, controlled trial. *Gastroenterology* 2014; 147: 1327-1337.
28. Rincon D, Vaquero J, Hernando A, *et al.* Oral probiotic VSL#3 attenuates the circulatory disturbances of patients with cirrhosis and ascites. *Liver Int* 2014; 34: 1504-1512.
29. Gupta N, Kumar A, Sharma P Garg V, Sharma BC, Sarin SK. Effects of the adjunctive probiotic VSL#3 on portal haemodynamics in patients with cirrhosis and large varices: a randomized trial. *Liver Int* 2013; 33: 1148-1145.
30. Muir AJ. Understanding the complexities of Cirrhosis. *Clin Ther* 2015; 37: 1822-1836.
31. Bubnov RV, Spivak MY, Lazarenko LM, Bomba A, Boyko NV. Probiotics and immunity: provisional role for personalized diets and disease prevention. *EPMA* J 2015; 6: 14. doi: 10.1186/s13167-015-0036-0
32. Wunsch E, Post M, Gutkowski K, *et al*. Critical flicker frequency fails to disclose brain dysfunction in patients with primary biliary cirrhosis. *Dig Liver Dis* 2010; 42: 818-821.
33. Wunsch E, Koziarska D, Milkiewicz M, *et al*. In patients with liver cirrhosis, proinflammatory interleukins correlate with health-related quality of life irrespective of minimal hepatic encephalopathy. *Eur J Gastroenterol Hepatol* 2013; 25: 1402-1407.
34. Wunsch E, Naprawa G, Koziarska D, Milkiewicz M, Nowacki P, Milkiewicz P. Serum natremia affects health-related quality of life in patients with liver cirrhosis: a prospective, single centre study. *Ann Hepatol* 2013; 12: 448-455.
35. Bolognesi M, Di Pascoli M, Verardo A, GattaA. Splanchnic vasodilation and hyperdynamic circulatory syndrome in cirrhosis. *World J Gastroenterol* 2014; 220: 2555-2563.
36. Houde M, Desbiens L, D'Orleand-Juste P. Endothelin-1: biosynthesis, signaling and vasoreactivity. *Adv Pharmacol* 2016; 77: 143-175.
37. Angus W. Role of endothelin in systemic and portal resistance in cirrhosis. *Gut* 2006; 55: 1230-1232.
38. Vaughan RB, Angus PW, Chin-Dusting JP. Evidence for altered vascular responses to exogenous endothelin-1 in patients with advanced cirrhosis with restoration of the normal vasoconstrictor response following successful liver transplantation. *Gut* 2003; 52: 1505-1510.
39. Wereszczynka-Siemiatkowska U, Swidnicka-Siergiejko A, Siemiatkowski A, *et al.* Endothelin 1 and transforming growth factor-β1 correlate with liver function and portal pressure in cirrhotic patients. *Cytokine* 2015; 76: 144-151.
40. Rockey DC. Vascular mediators in the injured liver. *Hepatology* 2003; 37: 4-12.
41. Bellot P, Garcia-Pagan JC, Frances R, *et al.* Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. *Hepatology* 2010; 52: 2044-2052.
42. Ehling J, Bartneck M, Wei X, *et al.* CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. *Gut* 2014; 63: 1960-1971.
43. Kawano M, Miyoshi M, Ogawa A, Sakai F, Kadooka Y. Lactobacillus gasseri SBT2055 inhibits adipose tissue inflammation and intestinal permeability in mice fed a high-fat diet. *J Nutr Sci* 2016; 5: e23. doi: 10.1017/jns.2016.12
44. Dabos KJ, Yung DE, Bartzis L, Hayes PC, Plevris JN, Koulaouzidis A. Small bowel capsule endoscopy and portal hypertensive enteropathy in cirrhotic patients: results from a tertiary referral centre. *Ann Hepatol* 2016; 15: 394-401.
45. Koulaouzidis A, Ritchie G, Plevris JN. Portal hypertensive enteropathy in small-bowel capsule endoscopy. *Clin Gastroenterol Hepatol* 2012; 10: e54-e55. doi: 10.1016/j.cgh.2011.12.025.
46. Holzberg JR, Jin R, Le NA, *et al.* Plasminogen activator inhibitor-1 predicts quantity of hepatic steatosis independent of insulin resistance and body weight. *J Pediatr Gastroenterol Nutr* 2016; 62: 819-823.
47. Liu Q, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology* 2004; 39: 1441-1449.
48. McGee RG, Bakens A, Wiley K, Riordan SM, Webster AC. Probiotics for patients with hepatic encephalopathy. *Cochrane Database Syst Rev* 2011; 11: CD008716. doi: 10.1002/14651858.CD008716.pub2.
49. Li F, Duan K, Wang C, McClain C, Feng W. Probiotics and alcoholic liver disease: treatment and potential mechanisms. *Gastroenterol Res Pract* 2016; 2016: 5491465. doi: 10.1155/2016/5491465
50. Boryczka G, Hartleb M, Rudzki K, Janik MA. Influence of an upright body position on the size of intrapulmonary blond shunts in patients with advanced liver cirrhosis. *J Physiol Pharmacol* 2015; 66: 855-861.
51. Shanahan F, Quigley EM. Manipulation of the microbiota for treatment of IBS and IBD-challenges and controversies. *Gastroenterology* 2014; 146: 1554-1563.
52. Obata Y, Pachnis V. The effect of microbiota and the immune system on the development and organization of the enteric nervous system. *Gastroenterology* 2016; 151: 836-844.
53. Konturek PC, Haziri D, Brzozowski T, *et al.* Emerging role of fecal microbiota therapy in the treatment of gastrointestinal and extra-gastrointestinal diseases. *J Physiol Pharmacol* 2015; 66: 483-491.
54. Marlicz W, Loniewski I, Grimes DS, Quigley EM. Nonsteroidal anti-inflammatory drugs, proton pump inhibitors, and gastrointestinal injury: contrasting interactions in the stomach and small intestine. *Mayo Clin Proc* 2014; 89: 1699-709.
55. Dylag K, Hubalewska-Mazgaj M, Surmiak M, Szmyd J, Brzozowski T. Probiotics in the mechanism of protection against gut inflammation and therapy of gastrointestinal disorders. *Curr Pharm Des* 2014; 20: 1149-1155.
56. Konturek PC, Brzozowski T, Konturek SJ. Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options. *J Physiol Pharmacol* 2011; 62: 591-599.
57. Horvath A, B. Leber B, Schmerboeck B, *et al.* Randomised clinical trial: the effects of a multispecies probiotic vs. placebo on innate immune function, bacterial translocation and gut permeability in patients with cirrhosis. *Aliment Pharmacol* Ther 2016; 44: 926-935.
58. Elzouki AN. Probiotics and liver disease: where are we now and where are we going? *J Clin Gastroenterol* 2016; 50 (Suppl. 2): S188-S190.
59. Celinski K, Konturek PC, Slomka M, *et al.* Effects of treatment with melatonin and tryptophan on liver enzymes, parameters of fat metabolism and plasma levels of cytokines in patients with non-alcoholic fatty liver disease - 14 months follow up. *J Physiol Pharmacol* 2014; 65: 75-82.
60. Kalman RS, Goldberg DS. The role of obeticholic acid in gut bacterial translocation and inflammation. *Gastroenterology* 2016; 151: 759-761.
61. Schwabl P, Hambruch E, Seeland BA, *et al.* The FXR agonist PX20606 ameliorated portal hypertension by targeting

vascular remodeling and sinusoidal dysfunction. *J Hepatol* 2016; Dec. 16: doi: 10.1016/j.jhep.2016.12.005
62. Serino M, Blasco-Baque V, Nicolas S, Burcelin R. Managing the manager: gut microbes, stem cells and metabolism. *Diabetes Metab* 2014; 40: 186-190.
63. Monsour HP, Quigley EM. The microbiome: what will the future hold ? *Semin Liver Dis* 2016; 36: 354-359.
64. Acharya C, Betrapally NS, Gillevet PM, *et al.* Chronic opioid use is associated with altered gut microbiota and predicts readmissions in patients with cirrhosis. *Aliment Pharmacol Ther* 2017; 45: 319-331.
65. Yan K, Garcia-Tsao G. Novel prevention strategies for bacterial infections in cirrhosis. *Expert Opin Pharmacother* 2016; 17: 689-701.

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