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Rifaximin has minor effects on bacterial composition, inflammation, and bacterial translocation in cirrhosis: A randomized trial

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Author contributions: N. K., A. K., F. B., and S. M. developed the trial design and protocol. N. K., J. S. P., T. M. B., L. H., and S. M. performed trial-related procedures, and members of CoRif study group identified and referred eligible patients. J. T. analysed inflammation markers and performed quality validation on data and analyses. J. C. analysed bacterial DNA in blood and designed the graphical display and figures. M. S. M., W. A. A.-S., and S. J. S. analysed bacterial DNA in feces. N. K., J. C., M. S. M., and F. B. analysed and interpreted data. N. K. and F. B. drafted the first edition of the manuscript. All authors critically revised the manuscript and approved the final version.

Abstract

Background and Aim: Decompensated cirrhosis is characterized by disturbed hemodynamics, immune dysfunction, and high risk of infections. Translocation of viable bacteria and bacterial products from the gut to the blood is considered a key driver in this process. Intestinal decontamination with rifaximin may reduce bacterial translocation (BT) and decrease inflammation. A randomized, placebo-controlled trial investigated the effects of rifaximin on inflammation and BT in decompensated cirrhosis.

Methods: Fifty-four out-patients with cirrhosis and ascites were randomized, mean age 56 years (± 8.4), and model for end-stage liver disease score 12 (± 3.9). Patients received rifaximin 550-mg BD ($n = 36$) or placebo BD ($n = 18$). Blood and fecal ($n = 15$) sampling were conducted at baseline and after 4 weeks. Bacterial DNA in blood was determined by real-time qPCR 16S rRNA gene quantification. Bacterial composition in feces was analyzed by 16S rRNA gene sequencing.

Results: Circulating markers of inflammation, including tumor necrosis factor alpha, interleukins 6, 10, and 18, stromal cell-derived factor 1-α, transforming growth factor β-1, and high sensitivity C-reactive protein, were unaltered by rifaximin treatment. Rifaximin altered abundance of bacterial taxa in blood marginally, only a decrease in *Pseudomonadales* was observed. In feces, rifaximin decreased bacterial richness, but effect on particular species was not observed. Subgroup analyses on patients with severely disturbed hemodynamics ($n = 34$) or activated lipopolysaccharide binding protein ($n = 37$) revealed no effect of rifaximin.

Conclusion: Four weeks of treatment with rifaximin had no impact on the inflammatory state and only minor effects on BT and intestinal bacterial composition in stable, decompensated cirrhosis (NCT01769040).

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Introduction

Bacterial infections are frequent complications in cirrhosis. They often precipitate decompensation and substantially influence survival.[1] Infections may be triggered by translocation of bacteria and bacterial products from the gut into the lymph and bloodstream.[2,3] In the stable but decompensated patient with cirrhosis, the inflammatory activity is enhanced.[4] Bacterial products enhance tumor necrosis factor alpha (TNFα) production and toll-like receptor 4 signaling pathways, which leads to increased cytokine release.[5–7] The cytokines induce expression of nitrogen oxidative species that are involved in vasodilation and endothelial dysfunction in arterioles.[8] Lipopolysaccharide binding protein (LBP), a surrogate marker of endotoxemia, as well as high levels of interleukin-6 (IL-6) and TNFα are associated with a lower systemic vascular resistance (SVR) and a higher cardiac output in cirrhosis.[9–12]

Oral broad-spectrum antibiotics have shown efficacy in preventing bacterial translocation (BT) and improving the deranged systemic hemodynamics.[9,13,14] Rifaximin is a non-absorbable antibiotic, with broad spectrum effects on both Gram-positive and Gram-negative bacteria.[15,16] It is approved for the prevention of recurrent hepatic encephalopathy (HE).[17,18] Studies have investigated the effects of rifaximin on endotoxin levels in cirrhosis.[19,20] A single study found a decrease in endotoxins but no significant alterations in gut microflora after rifaximin treatment.[21] Alterations in favor of less pathogenic species such as *Lactobacillus* species and suppression of the families *Clostridiaceae* and *Peptostreptococcaceae* have been demonstrated in rats.[22] However, the mechanisms of action of rifaximin in cirrhosis remain elusive.

We aimed to assess the effects of rifaximin on BT and the inflammatory state in patients with cirrhosis and ascites in a randomized clinical trial.

Methods

The trial was registered accordingly (EudraCT 2012-002890-71 and NCT01769040), and the Scientific Ethics Committee of the Capital Region of Denmark approved the trial (H-1-2012-078). The Good Clinical Practice Unit, Copenhagen University monitored the trial. All patients gave informed written consent.

Patients. Between February 2013 and December 2015, 54 patients with decompensated cirrhosis were included in a double-blind, randomized, and controlled trial.

Inclusion and exclusion criteria, as well as trial design and endpoints have been described in detail previously.[23] In brief, patients were randomized to either rifaximin 550 mg twice daily or identical placebo for 4 weeks. All trial investigations were performed blinded.

Methods. Medical history and standard biochemical lab tests were taken on the day of inclusion. The complete investigational program was performed at baseline and after 4 weeks of treatment.[23] The program included, as previously described, assessment of hemodynamics with liver vein catheterization and characterization of patients with kidney function and hepatic encephalopathy.[23] During sterile conditions at liver vein catheterization, 25 mL of blood was drawn from the femoral artery. Samples of whole blood and EDTA plasma were stored at −80 °C until analysis. Fifteen of the 54 participants delivered a fecal sample at baseline, and at follow up.

Analysis of inflammation markers. High sensitivity C-reactive protein (hs-CRP), stromal cell-derived factor 1 alpha (SDF-1α), transforming growth factor beta 1, interferon gamma-induced protein 10, interleukins 10 and 18 (IL-10 and IL-18) were analyzed with a commercially available enzyme-linked immunosorbent assay (Quantikine, R&D Systems Europe, Ltd. Abingdon OX14 3NB, UK). For hs-CRP, sensitivity was 0.022 ng/mL, and the coefficient of variation (CV) was 5.3%. For SDF-1α, transforming growth factor beta 1, interferon gamma-induced protein 10, IL-10, and IL-18, sensitivities were 4.7, 15.4, 4.5, 3.9, and 7.5 pg/mL, respectively, and CVs were 2.5%, 3.8%, 2.8%, 16.6%, and 4.6%, respectively.[24]

The cytokines IL-6, TNFα, IL-1β, and IL-4 were analyzed with a high sensitivity Luminex assay, (Magnetic Luminex Performance, R&D Systems Europe, Ltd., Abingdon OX14 3NB, UK). Less than 0.5% cross-reactivity and interference between agents tested were seen. Sensitivities for IL-6, TNFα, IL1β, and IL-4 were 0.7, 5.5, 1.0, and 10 pg/mL, respectively, and CVs were 6.6%, 7.1%, 12.9%, and 8.4%, respectively.[24]

Measurement of endotoxin markers. Lipopolysaccharide (LPS), LBP, and the functional receptor for LPS, soluble CD14 (sCD14), were measured in EDTA plasma. LPS levels were determined using the Limulus amoebocyte lysate kinetic chromogenic methodology, optimized for sensitivity (Vaiomer SAS, Toulouse, France) with a commercially available kit (Charles River).[25,26] The quantifiable limit for LPS was 0.024 EU/mL. The quantifiable limits for LBP and sCD14 were 3.5 and 0.13 μg/mL, respectively.

Quantitation and sequencing of bacterial DNA from whole blood. Extraction methods have been extensively described previously.[27,28] Bacterial DNA (BDNA) was isolated from whole blood with a DNA isolation tool (NucleoSpin blood kit; Macherey-Nagel, Düren, Germany) and assessed by electrophoresis and spectrophotometry (NanoDrop, Thermo Fisher Scientific, USA).

16S rRNA gene quantification was determined by real-time qPCR, using primers that target the V3-V4 hypervariable regions of the 16S ribosomal gene with a sensitivity of 95% and a specificity of 100% (Vaiomer universal 16S primers; Vaiomer SAS, Toulouse, France). Quantity of BDNA is reported as 16S copies per ng of total DNA.

A sequencing library was generated for each sample by PCR amplification of the bacterial 16S rRNA gene V3-V4 region using universal primers (Vaiomer universal 16S primers) and addition of sequencing adapters. The 476 base pair amplicon products were sequenced using 2 × 300 base pair paired-end Illumina MiSeq sequencing methodology with reagent kit v3. After demultiplexing of the barcoded Illumina reads, single read sequences were removed and paired reads joint into complete fragments. Following quality-filtering (abundance, fragment length, and sample quality), the sequences were clustered into operational taxonomic units with a 95% identity threshold, aligned against a 16S rRNA gene reference database for taxonomic assignment, and then the community profiles were determined.

Analysis of feces microbiota composition. Sequencing libraries were prepared using a two-step PCR protocol,[29] modified to amplify both variable region V3 and V4 of the 16S rRNA gene, approximately 460 bp. In the first step, general primers were used,[30,31] and in the second step, the primers additionally included sequencing adaptors and barcode tags. Purification and sequencing were performed as previously described.[30,31]

MiSeq Controller Software was used for sequence demultiplexing. Sequence mate-pairing and filtering was done using USEARCH v7.0.1090.[32] Operational taxonomic units clustering, dereplication, and singleton removal was performed using UPARSE.[33] Chimera checking and removal was performed using USEARCH and the ChimeraSlayer package.[34] A phylogenetic tree was built with QIIME wrappers for PyNAST, *FastTree*, and alignment filtering.[35–37]

Statistical analyses. Differences between the two groups were assessed as unpaired *T*-testing of delta values and as control by analysis of variance (repeated measurements ANOVA). Data were controlled for normal distribution and transformed accordingly. The linear discriminant analysis effect size (LefSe) is an algorithm for high-dimensional biomarker discovery, emphasizing both statistical significance and biological relevance.[38] LefSe was determined using default values (alpha value 0.5 and threshold 2.0 for logarithmic linear discriminant analysis score for discriminative features) and the strategy for multi-class analysis set to "all-against-all." Data were handled using SAS statistical software (v9.4 and Enterprise v7.1; SAS Institute, North Carolina, USA) and GraphPad Prism (v 6.0.7). The microbiota data were analyzed using the physeq package in R. To describe the beta diversity, we used multivariate analysis of variance using distance matrices (PERMANOVA).

Results

Forty-five men and nine women with a median age of 56 years (33–74) were randomized. Baseline characteristics are summarized in Table 1. Flow of patients and demographics are described in detail earlier.[23]

After 6 months of follow up, 10 patients had died (six in the rifaximin group and four in the placebo group). There were five episodes of HE (four in the rifaximin group and one in the placebo group) and four episodes of severe infection (two episodes of spontaneous bacterial peritonitis in the rifaximin group and two episodes of pneumonia in the placebo group). Rifaximin did not improve risk of HE or infections (P = 0.50 and P = 0.90, respectively).

Inflammation markers. In more than 85% of samples, the level of cytokines IL-1β, IL-4, and IL-10 were below the lowest quantifiable limit, suggesting a minimal activation of these cytokines in patients with stable decompensated cirrhosis and no clinical infection (Table 2). TNFα, TGF-1β, and SDF-1α were increased above normal range in $>$ 90% of samples, and hs-CRP was elevated in 55% of samples. Rifaximin had no significant impact on any of the cytokine levels compared with placebo after 4 weeks of treatment. TNFα, TGF-1β, hs-CRP, and SDF-1α were not associated with Child score or model for end-stage liver disease score, or presence of minimal hepatic encephalopathy (MHE). No correlation to the hemodynamic parameters hepatic venous pressure gradient, SVR, cardiac output, and central blood volume could be demonstrated.

Table 1 Patient characteristics at inclusion

	Rifaximin (n = 36)	Placebo (n = 18)	P-value
Age	58.5 (33–68)	52.5 (34–74)	0.2
Sex (male/female)	31/5	14/4	NA
Child class B/C	27/9	17/1	NA
MELD score	12 (6–25)	9.5 (6–15)	0.02
Biochemistry			
Hemoglobin mmol/L	7.6 (5.3–9.6)	7.9 (5–9.8)	0.36
WBC 10^5/L	6.3 (2.6–13.2)	7.1 (3.6–16.9)	0.38
Platelets 10^9/L	131 (27–562)	152 (56–275)	0.88
CRP U/L	5 (0.3–40)	5 (0–31)	0.94
MHE at baseline	22	12	NA
Previous episodes of HE	8	2	NA
PHES	−6 (−13–3)	−7 (−15–2)	0.46
HVPG mmHg	17 (11–26)	15 (12–27)	0.73
Cardiac output L/min	6.7 (4.2–11.8)	6.0 (3.83–10.10)	0.58
Systemic vascular resistance dynes × cm^5/min	934 (568–1849)	941 (657–1901)	0.46
Glomerular filtration rate mL/min	87.0 (26.4–127.1)	78.6 (34.4–142.9)	0.93

Data are given in median and total range. MHE is defined as PHES score less than −4.

CRP, C-reactive protein; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease; MHE, minimal hepatic encephalopathy; PHES, psychometric hepatic encephalopathy score; WBC, white blood cell count.

Table 2 Comparison of baseline and follow-up levels of cytokines between groups

Inflammation markers	Rifaximin (n = 36)		Placebo (n = 18)		P-value	Normal range[†]	LQL[‡]
	Baseline	Follow up	Baseline	Follow up			
TNFα pg/mL	8.81 (4.13)	8.24 (3.81)	11.22 (8.23)	7.57 (3.66)	0.57	0.00–1.60	1.60
IL-4 pg/mL[§]	NA	NA	NA	NA	—	0.00–0.25	0.25
IL-6 pg/mL	14.107 (33.823)	5.753 (7.396)	7.325 (6.508)	6.665 (6.273)	0.16	0.7–12.5	0.70
IL-10 pg/mL	0.483 (0.630)	0.325 (0.470)	0.481 (0.644)	1.043 (1.717)	0.08	0.0–0.78	0.78[¶]
IL-18 pg/mL	432.9 (485.4)	406.4 (492.5)	448.6 (667.9)	351.5 (318.3)	0.26	2102–6718	2.25
IP10 pg/mL[¶]	265.1 (166.3)	246.6 (107.5)	319.3 (250.6)	343.8 (365.9)	0.45	47–382	1.67
IL1β pg/mL[††]	0.187 (0.236)	0.155 (0.218)	0.255 (0.149)	0.248 (0.219)	0.61	0.0–3.9	3.9[¶]
SDF-1α pg/mL	3741 (746)	3687 (740)	3673 (508)	3718 (502)	0.18	1360–2900	18
Hs-CRP ng/mL	7647 (7980)	6910 (8147)	8067 (6992)	7172 (8132)	0.87	104–4185	0.01
TGF-1β pg/mL	12221 (10413)	11852 (8741)	10089 (5898)	9278 (6093)	0.70	903–1654	4.61

[†]Normal range provided by R&D systems, manufacturer of ELISA and Luminex kits. Tested in healthy volunteers (n = 10–40). Normal range below LQL indicates a minimal or zero production of these cytokines in healthy subjects.
[‡]LQL, lowest quantifiable limit, standard test in 40 assays.
[§]IL-4 was below detection limit in 53 patients (35 rifaximin group/18 placebo group) (101 samples).
[¶]IL-10 was below detection limit in 22 patients (18 rifaximin group/four placebo group) (36 samples)
[††]IL1β was below detection limit in one patient (rifaximin group) (two samples).
All values are given in mean and SD unless otherwise stated. Unpaired T-test is performed on delta values, defined as follow up minus baseline. Markers reflecting inflammation by cytokine expression: IL-6, interleukin 6; IL-10, interleukin 10; Hs-CRP, high-sensitivity C-reactive protein; TNFα, tumor necrosis factor alpha. Markers that reflects activation of inflammatory cells and may affect stellate cells in the liver: IL1β, interleukin 1 beta (related to acute inflammation); IL-4, interleukin 4; IL-18, interleukin 18; IP10, interferon gamma-induced protein 10 (correlated to formation of liver cirrhosis); SDF-1α, stromal cell-derived factor 1-alpha; TGF-β1, transforming growth factor beta 1.

Endotoxin markers. The levels of LPS remained unaltered after treatment (rifaximin group, mean ± SD 0.089 μg/mL ± 0.049 at baseline *vs* 0.095 EU/mL ± 0.088 at follow up, compared with placebo group, 0.089 μg/mL ± 0.044 at baseline *vs* 0.113 μg/mL ± 0.122 at follow up, P = 0.16). LBP was elevated above 5.9 μg/mL in 37 patients and decreased with rifaximin treatment as reported previously.[23] Likewise, levels of sCD14 were elevated, but there were no differences between groups (rifaximin group, mean ± SD 1.57 μg/mL ± 0.53 at baseline *vs* 1.58 μg/mL ± 0.58 at follow up, compared with placebo group, 1.65 μg/mL ± 0.41 at baseline *vs* 1.68 μg/mL ± 0.39 at follow up) (Fig. 1).

Bacterial DNA in blood. Bacterial DNA was found in all 103 samples analyzed. On order and family level, approximately 35% was derived from gut-associated bacteria (e.g., *Bacteroidetes*, *Bifidobacteriae*, and *Pseudomonadaceae*). Skin microbiota and environmental bacteria amounted to 52% (e.g., *Propionibacteriaceae*, *Corynebacteriaceae*, *Micrococaceae*, and *Methylobacteriaceae*). BDNA from 240 different bacterial genera were detected, with a median of 20 genera representing at least 1% of total BDNA in each patient. When differences in abundance between groups were assesed, a trend towards decreased abundance of the class *Gammaproteobacteria* and order *Pseudomonadales* was seen after treatment with rifaximin, as well as an increase of the phylum Actinobacteria (Fig. 2). The two genera *Lactobacillus* and *Methylobacterium* were very abundant in all samples (relative abundance between 32.1% and 7.5%). To determine if these genera might have hidden differences of less abundant bacteria, a LefSe analysis was generated for all data (data not shown), and without *Lactobacillus* and *Methylobacterium*. Only two significant differential features were identified in the comparison, indicating that the presence of the *Lactobacillus* and *Methylobacterium* signatures had minimal impact on the LEfSe differential abundance analysis (Fig. 2). On class level, there was a relative increase in Actinobacteria in the rifaximin group, as well as a decrease of *Pseudomonadales* on order level.

The total amount of 16S copies/ng of DNA in blood was similar in both treatment groups and unaltered by treatment. Overall, 4 weeks of rifaximin treatment altered relative abundance of BDNA in blood minimally (Figure S1). At phylum level, there was a trend towards an increase in Proteobacteria in rifaximin group, and at order level, a trend towards an increase in Bifidobacteriales after rifaximin therapy. We also analyzed numerical difference in ng BDNA from specific bacterial classes, orders, families, and genera, but no significant difference between baseline and follow up was detected in any of these bacteria, across the taxonomic levels (Table S1).

Microbiota composition in feces. Of the 30 samples sequenced (11 sample pairs from rifaximin group and four sample pairs from the placebo group), three samples were excluded due to low sequence read counts (less than 2000 reads). The remaining 27 samples had in average 10 435 reads (SD = 3888 reads). No alpha diversity, observed richness, and Shannon diversity index, differences were observed between rifaximin and placebo groups at baseline. No significant differences in richness were observed at follow up, but Shannon diversity index were lower in the rifaximin-treated group at follow up (rifaximin group, mean ± SD 2.78 ± 0.30 *vs* placebo group, 3.05 ± 0.11, P = 0.033) (Fig. 3).

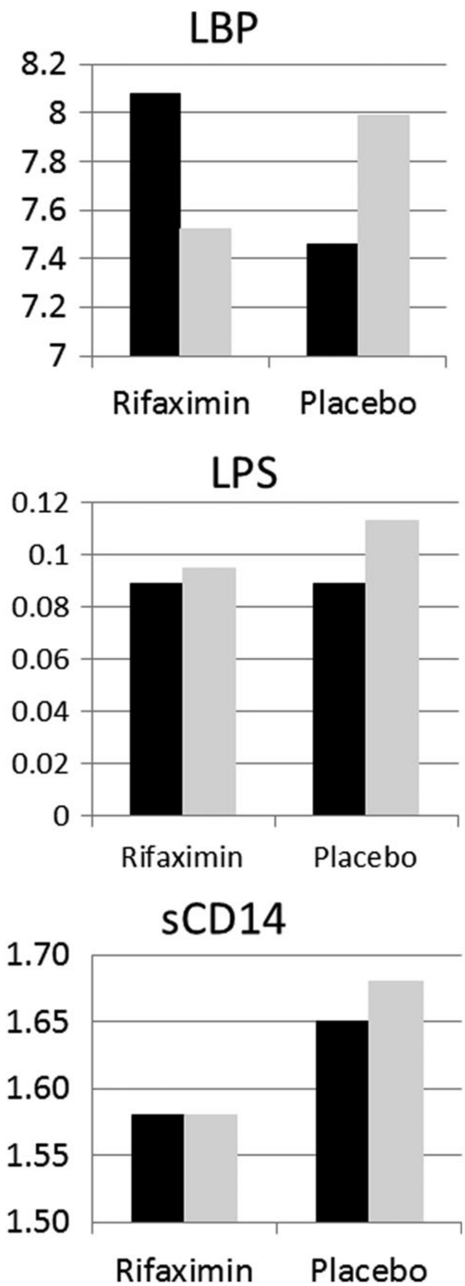

Figure 1 Change in LBP, LPS, and sCD14 levels in rifaximin and placebo groups. LBP, lipopolysaccharide binding protein (μg/mL); LPS, lipopolysaccharides (EU/mL); sCD14 (μg/mL). There was a significant difference in LBP (P = 0.02), but not in LPS and sCD14 (P = 0.16 and P = 0.9, respectively) between rifaximin and placebo groups. ■, baseline; ■, follow up.

The beta diversity (Bray–Curtis dissimilarity) did not show any clustering by either treatment or between baseline and follow up samples. When comparing the dissimilarity between samples from each individual, the rifaximin group had a larger separation between samples (rifaximin group, mean ± SD 0.38 ± 0.08 *vs* placebo group, 0.32 ± 0.02, P = 0.034). No genera had significantly different relative abundances when comparing baseline and follow up in the rifaximin group or between rifaximin and placebo groups at follow up.

Discussion

The main findings of the present study were that rifaximin treatment had no effect on activated inflammation markers TNFα, SDF-1α, TGF-1β, and hs-CRP and did not change LPS and sCD14. All patients had bacterial translocation from the gut. However, rifaximin had limited impact on the amount and composition of BDNA in blood. In feces, rifaximin decreased diversity of BDNA. A link between BDNA and increased inflammation markers could not be established. To our knowledge, this is the first interventional study to assess BDNA in blood and feces in the same cohort.

Bacterial translocation and activated LBP have been linked to dysfunctional circulation with low SVR and increased renin activity in one study of 102 cirrhotic patients.[9] Our study cohort had a hemodynamic profile similar to this, and 37 patients had activated LBP.[23] Association between markers of BT and activation of inflammatory cytokines has been demonstrated in previous studies,[9,26] but the exact value of identifying bacterial products and BDNA in the blood in relation to factors of inflammation or immune dysfunction remains controversial,[39–41]

This lack of effect on inflammation markers was similarly demonstrated in a randomized trial comparing rifaximin with placebo for MHE.[42] Some impact of treatment on TNFα and IL-6 would have been expected because these markers are directly activated through toll-like receptor 4 that responds to pathogen-associated molecular patterns from the gut.[43]

Inflammation markers are not readily used in clinics, and only a few studies in larger cohorts of both healthy subjects and patients with liver disease have been published.[44,45]

Modulation of the gut microbiome has previously been evaluated in a small cohort study of 20 patients with cirrhosis and MHE.[21] A significant decrease in *Veillonellaceae* and *Eubacteriaceae* of the fecal microbiome was seen after rifaximin treatment, but no changes in overall abundance of bacteria or bacterial load were found. Bacterial remnants from *Veillonellaceae* were not detected in our cohort, and the family *Eubacteriaceae* was unaltered by treatment. Bacterial load and specimens in blood and feces are not expected to be fully in agreement, and the impact of these differences is not clearly understood.[46] BT from the gut may not be the only source of bacteria in blood, as studies have shown high similarity between bacteria in blood and the oral microbiome.[47] This will clearly cause great discrepancy between results from blood and feces, as recently described.[45] Also, various methods and materials for extracting BDNA has been applied.[9,26,28,45] Cautiousness should therefore be applied when interpreting results.

In our material, all samples were positive for BDNA. More than 80% of BDNA was classified to family or genus level. These findings differ from earlier trials, where BDNA was detected in less than a third of blood and ascites samples, and no link to active infection or survival could be established.[26,40,41,48] The applied PCR-dependent methods detect BDNA in healthy individuals and patient cohorts where BT is not suspected, but in varying

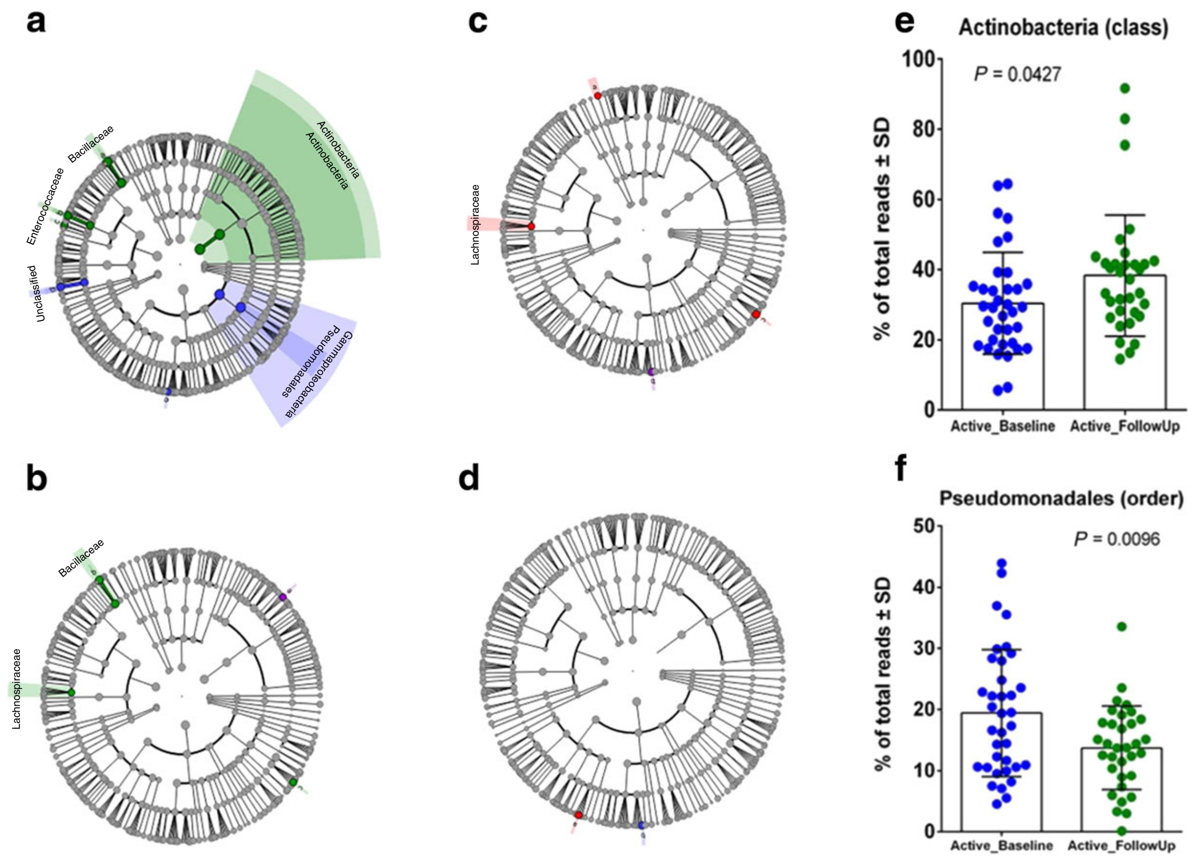

Figure 2 Differential abundances of bacterial taxa in whole blood. Cladograms derived from pairwise group LEfSe analysis of 16S rRNA gene sequences from whole blood: (a), active baseline versus, active follow up (, A: Bacillus; , B: Enterococcus; , C: Lactococcus; , D: unclassified; , E: Comamonas); (b), active follow up versus, placebo follow up (, A: Marmoricola; , B: Basillus; , C: Enhydrobacter); (c), placebo baseline versus, placebo follow up (, A: unclassified; , B: unclassified; , C: Enhydrobacter); and (d), active baseline versus, placebo baseline (, A: Sphingomonas; , B: unclassified). The cladograms show the taxonomic levels represented by rings with phyla at the innermost ring and genera at the outermost ring, and each circle is a member within that level. ($P < 0.05$; LDA score > 3.5). Differential feature histograms for (e) Actinobacteria (phylum) and (f) Pseudomonadales (order) of active baseline versus active follow up.

amounts and with great variance between individuals.[27,28] The variation of bacteria was substantial, and further sub-analyses on genus levels induced even greater variation between individual samples. A diversified microbiome exists in both healthy and severely ill patients, and the physiological mechanisms and impact of this remain to be clarified. We found no beneficial effect of rifaximin on clinical outcomes; however, the trial was not designed to address this properly, the sample size was limited, and treatment period was rather short. Future trials should assess the impact of changes in gut flora on clinically relevant outcomes.

The strengths of this manuscript are the rigorous methodology applied and the randomized design that is suitable for assessing effects of rifaximin on BT and inflammation. The great variation in BT and gut flora makes the trial underpowered when assessing associations between abundance of bacteria and single cytokines, as well as the small number of paired fecal samples makes it difficult to compare BDNA in blood and stool.

This is the first study to address the impact of rifaximin on BDNA in blood in a randomized, controlled trial. We investigated the effects of rifaximin in the stable, decompensated patient without signs of acute infection or acute-on-chronic liver failure. These patients have an ongoing BT and elevated LBP but no clinical signs of infection. Other trials have investigated the systemic inflammation theory in patients with severe systemic comorbidity, liver failure, and systemic inflammatory response syndrome.[49,50] It is likely that rifaximin exerts more pronounced effects in these patient categories. Although all patients were decompensated and had ascites, only 10 patients were Child class C. Systemic inflammation is more prone in progressed decompensation.[44]

The systemic inflammation theory has changed the paradigm of understanding systemic liver disease and provides a substantial and plausible explanation to the systemic mechanisms involved in development of critical events in cirrhosis.[44] However, BT may not exert substantial impact on hemodynamics in the "stable" decompensated patient. Rifaximin has no impact on inflammation and only exerts minor changes on bacterial composition in both blood and feces.

Future trials in this area should address the impact of antibiotic prophylaxis in a longer period after an episode of infection or acute decompensation in cirrhosis with focus on prevention of re-hospitalization, morbidity, and mortality in this challenging patient group.

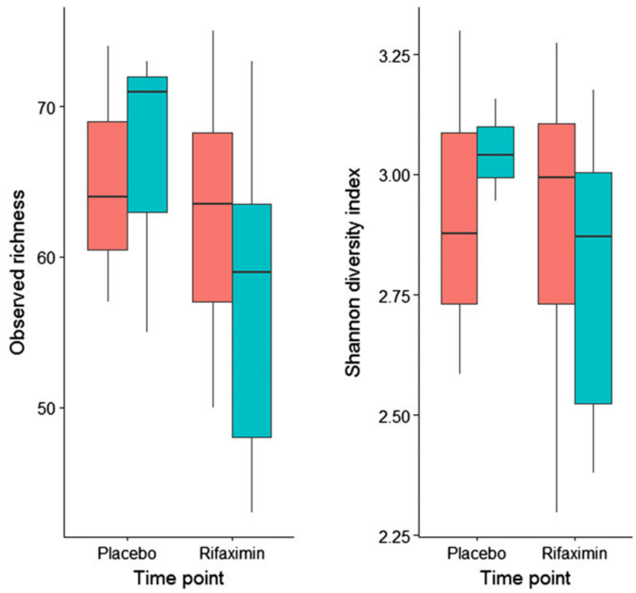

Figure 3 Observed richness of bacteriae in gut flora. Time point: , baseline; , follow up.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1. Relative abundance of taxons at the ‘order’ level. Average over groups.
Table S1. Comparison of baseline and follow-up load of DNA from bacterial sources.