Rifaximin has minor effects on bacterial composition, inflammation and bacterial translocation in cirrhosis

A randomized trial

Kimer, Nina; Pedersen, Julie S; Tavenier, Juliette; Christensen, Jeffrey E; Busk, Troels Malte; Hobolth, Lise; Krag, Aleksander; Al-Soud, Waleed Abu; Mortensen, Martin S; Sørensen, Søren J.; Møller, Søren; Bendtsen, Flemming; And members of the CoRif study group

Published in:
Journal of Gastroenterology and Hepatology

DOI:
10.1111/jgh.13852

Publication date:
2018

Document version:
Accepted manuscript

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

Nina Kimer[1,2], Julie S Pedersen[1], Juliette Tavenier[3], Jeffrey E Christensen[4,5], Troels M Busk[2], Lise Hobolth[1,6], Aleksander Krag, [7], Waleed Abu Al-Soud[8], Martin S Mortensen[8], Søren J Sørensen[8], Søren Møller[2], Flemming Bendtsen[1]

And members of the CoRif study group.

1 Gastro Unit, Medical Division, Copenhagen University Hospital Hvidovre

2 Centre of Diagnostic Imaging and Research, Department of Clinical Physiology and Nuclear Medicine, Copenhagen University Hospital Hvidovre

3 Clinical Research Centre, Copenhagen University Hospital Hvidovre

4 Vaiomer SAS, Labege Toulouse, France

5 Institute of Cardiovascular and Metabolic Diseases (I2MC), INSERM U1048, Toulouse, France

6 Department of Gastroenterology and Hepatology, Copenhagen University Hospital Bispebjerg, Denmark

7 Department of Gastroenterology and Hepatology, Odense University Hospital; Institute of Clinical Research, University of Southern Denmark, Denmark.

8 Section of Microbiology, Department of Biology, University of Copenhagen, Copenhagen, Denmark.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jgh.13852

Correspondence to:

Nina Kimer

Gastro Unit, Medical division

Copenhagen University Hospital Hvidovre

Kettegaard Alle 30

2650 Hvidovre

Denmark

Tel: +45 38 62 31 81

Nina.kimer@regionh.dk

Clinical trial number: EudraCT 2012-002890-71 **/** NCT01769040

Figures: 4, Tables: 3

CONFLICTS OF INTEREST

NK and FB received study medication and placebo free of charge from Norgine Denmark A/S for the present trial. Norgine also supplied an unrestricted grant for analysis of bacterial DNA. Norgine has not been involved in trial design, conduction of trial or analysis of trial data. SM has received a supporting grant from Novo Nordisk foundation. FB and TB has received speaker honorarium from Norgine. AK is a member of an advisory board for Norgine and has received speaker honorarium from Norgine. JC is employed by Vaiomer SAS, Labege, France. JSP, MSM, WAA, SJS and JT declare no conflicts of interest.

FINANCIAL SUPPORT

This trial was funded by Research Foundation of The Capital Region of Denmark, Novo Nordisk Foundation, and Norgine Denmark A/S.

The Copenhagen Rifaximin (CoRif) study group:

Lise Lotte Gluud[a], Flemming Tofteng[a], Karin Grønbæk[a], Camilla Nøjgaard[a], Thomas Blixt[a], Ida Vind [a], Mille B Nielsen[a], Eva G Theisen[b], Lone G Madsen[c], Sanne Dam-Larsen[c], Srdan Novovic[a], Stine Sloth[d], Marianne Vester-Andersen[e], Christian O Mortensen[f]

a Gastro Unit, medical division, Copenhagen University Hospital Hvidovre, Denmark

b Department of Hepatology, Copenhagen University Hospital Rigshospitalet, Denmark

c Department of internal medicine, Zealand University Hospital Køge, Denmark

d Gastro Unit, Copenhagen University Hospital Herlev, Denmark

e Department of Gastroenterology and Hepatology, Copenhagen University Hospital Bispebjerg, Denmark

f Department of internal medicine, Copenhagen University Hospital Gentofte, Denmark

ABBREVIATIONS

TNFα: Tumour necrosis factor alpha; TLR4: toll-like receptor 4; LPB: lipopolysaccharide binding protein; IL-6: Interleukin-6; SVR: systemic vascular resistance; CO: cardiac output; BT: bacterial translocation; LVC: liver vein catheterization; BDNA: bacterial DNA; OTU: operational taxonomic units; HE: hepatic encephalopathy; MHE: minimal hepatic encephalopathy; HVPG: hepatic venous pressure gradient; CRT: continuous reaction time; PHES: psychometric hepatic encephalopathy score; hs-CRP: high-sensitivity C-reactive protein; SDF-1α: Stromal cell-derived factor 1-alpha; TGF-β1: transforming growth factor beta 1; IP10: interferon gamma induced protein-10; IL-10: interleukin-10; IL-18: interleukin-18; Il-1β: Interleukin-1 beta; IL-4: interleukin 4; LPS: lipopolysaccharide, MDD: minimal detectable dose; DST: digit symbol test; NCT-A and B: number connection test A and B; SDT: serial dotting test; LTT: line tracing test; LefSe: linear discriminant analysis effect size; LQL: lowest quantifiable limit; MELD: model for end-stage liver disease; ACLF: acute-on-chronic liver failure

ACKNOWLEDGEMENTS

We are indebted to bio-analysts Katrine Lyngby, Bente Pedersen, Department of Clinical Physiology and Nuclear Medicine, Copenhagen University Hospital Hvidovre, for handling biological material.

AUTHOR CONTRIBUTIONS

Nina Kimer (NK) is the guarantor of the manuscript. NK, Aleksander Krag, Flemming Bendtsen (FB) and Søren Møller (SM) developed the trial design and protocol. NK, JSP, Troels Malte Busk, Lise Hobolth and SM performed trial related procedures and members of CoRif study group identified and referred eligible patients. Juliette Tavenier analysed inflammation markers and performed quality validation on data and analyses. Jeffrey Christensen (JC) analysed bacterial DNA in blood and designed the graphical display and figures. Martin Steen Mortensen (MSM), Waleed Abu Al-Soud and Søren Johannes Sørensen analysed bacterial DNA in faeces. NK, JC, MSM, and FB analysed and interpreted data. NK and FB drafted the first edition of the manuscript. All authors critically revised the manuscript and approved the final version.

ABSTRACT

Background & aims: Decompensated cirrhosis is characterized by disturbed haemodynamics, 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. In 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 MELD score 12 (±3.9). Patients received rifaximin 550 mg BD (n=36) or placebo BD (n=18). Blood and faecal (n=15) sampling were conducted at baseline and after four weeks. Bacterial DNA in blood was determined by real-

time qPCR 16S rRNA gene quantification. Bacterial composition in faeces was analysed by 16S rRNA gene sequencing.

Results: Circulating markers of inflammation, including TNFα, interleukin-6, 10 and 18, Stromal cell-derived factor 1-α, transforming growth factor β-1 and high sensitivity CRP, were unaltered by rifaximin treatment. Rifaximin altered abundance of bacterial taxa in blood marginally, only a decrease in Pseudomonadales was observed. In faeces, rifaximin decreased bacterial richness, but effect on particular species was not observed. Subgroup analyses on patients with severely disturbed hemodynamics (n=34); or activated LBP (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).

Keywords: cytokines, inflammation, cirrhosis, bacterial translocation, portal hypertension

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 blood stream.[2, 3] In the stable, but decompensated patient with cirrhosis, the inflammatory activity is enhanced.[4] Bacterial products enhance tumour necrosis factor alpha (TNFα) production and toll-like receptor 4 (TLR4) signalling 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 (CO) 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 favour 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 randomised clinical trial.

PATIENTS AND 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.

In- 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 four 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 four weeks of treatment.[23] The programme included as previous described, assessment of hemodynamics with liver vein catheterization (LVC) and characterization of patients with kidney function and hepatic encephalopathy.[23] During sterile conditions at LVC, 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 faecal 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 (TGF-β1), interferon gamma induced protein 10 (IP10), interleukins 10 and 18 (IL-10 and IL-18) were analysed with a commercially available enzyme-linked immunosorbent assay (ELISA) (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, TGF-β1, IP10, IL-10 and IL-18, sensitivity was 4.7, 15.4, 4.5, 3.9 and 7.5 pg/ml, respectively, and CV was 2.5, 3.8, 2.8, 16.6, and 4.6%.[24]

The cytokines IL-6, TNFα, interleukin-1 beta (IL-1β), and interleukin-4 (IL-4) were analysed with a high sensitive 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. Sensitivity for IL-6, TNFα, IL1β and IL-4 was 0.7, 5.5, 1.0, and 10 pg/ml, respectively, and CV was 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 limit for LBP and sCD14 were 3.5 µg/ml 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).

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 x 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 (OTU) 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 faeces 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, approx. 460bp. 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] OTU 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 (alfa-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 was 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 analysed using the physeq package in R. To describe the beta diversity we used Multivariate Analysis of Variance Using Distance Matrices (PERMANOVA).

RESULTS

Forty-five males and 9 females 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 six months of follow-up, ten patients had died (six in the rifaximin group, four in the placebo group). There were 5 episodes of HE (four in the rifaximin group, 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 (LQL), 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 to placebo after

four weeks of treatment. TNFα, TGF-1β, hs-CRP, and SDF-1α were not associated with Child score or model for end-stage liver disease (MELD) score, or presence of MHE. No correlation to the hemodynamic parameters HVPG, SVR, CO and central blood volume could be demonstrated.

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 to 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 to placebo group, 1.65 µg/ml ±0.41 at baseline vs. 1.68 µg/ml ±0.39 at follow up) (Figure 1).

Bacterial DNA in blood. BDNA was found in all 103 samples analysed. On order and family level, approximately 35 % was derived from gut-associated bacteria (e.g. Bacteroidetes, Bifidobacteriae, and Pseudomonaceae). Skin microbiota and environmental bacteria amounted to 52% (e.g. Propionibacteriaceae, Corynebacteriaceae, Micrococaceae, Methylobacteriaceae etc.). 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 (Figure 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 (Figure 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 (Supplementary Figure SF1). 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 analysed 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 (Supplementary Table S1).

Microbiota composition in faeces. Of the 30 samples sequenced (eleven sample pairs from rifaximin group and four sample pairs from the placebo group), 3 samples were excluded due to low sequence read counts (less than 2,000 reads). The remaining 27 samples had in average 10,435 reads (sd = 3,888 reads). No alpha diversity, observed richness and Shannon diversity index (SDI), differences were observed between rifaximin and placebo groups at baseline. No significant differences in richness were observed at follow-up, but SDI 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) (Figure 3). 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 faeces, 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 faeces in the same cohort.

BT 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 haemodynamic profile similar to this, and thirty-seven 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 since these markers are directly activated through TLR4 which respond 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 faecal 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 faeces is 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 faeces, 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 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 remains 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 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, which 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 faecal 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 faeces.

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.

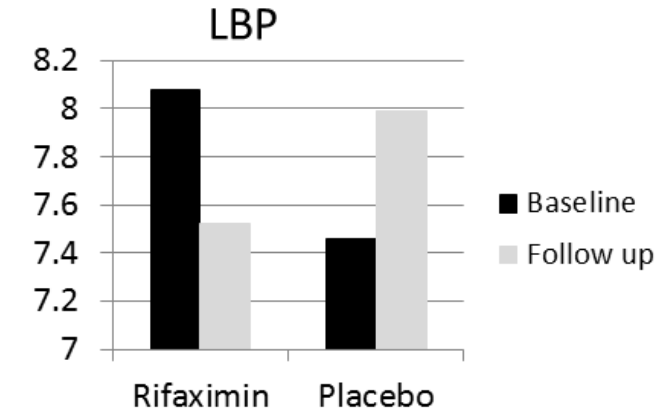
REFERENCES

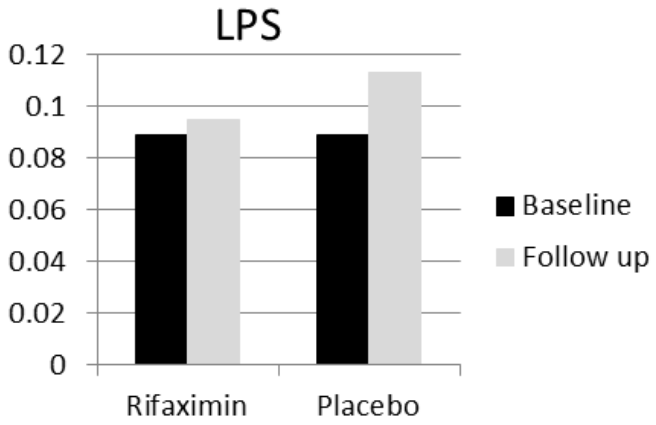
1. Fernandez J, Navasa M, Gomez J, et al. Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. Hepatology 2002;35:140-8.
2. Albillos A, Lario M, Alvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. J Hepatol 2014;61:1385-96.
3. Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. J Hepatol 2014;60:197-209.
4. Tilg H, Wilmer A, Vogel W, et al. Serum levels of cytokines in chronic liver diseases. Gastroenterology 1992;103:264-74.
5. Paik YH, Schwabe RF, Bataller R, et al. Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells. Hepatology 2003;37:1043-55.
6. Seki E, De Minicis S, Osterreicher CH, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med 2007;13:1324-32.
7. Tazi KA, Quioc JJ, Saada V, et al. Upregulation of TNF-alpha production signaling pathways in monocytes from patients with advanced cirrhosis: possible role of Akt and IRAK-M. J Hepatol 2006;45:280-9.
8. Hori N, Wiest R, Groszmann RJ. Enhanced release of nitric oxide in response to changes in flow and shear stress in the superior mesenteric arteries of portal hypertensive rats. Hepatology 1998;28:1467-73.
9. Albillos A, de la Hera A, Gonzalez M, et al. Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. Hepatology 2003;37:208-17.

10. Genesca J, Marti R, Rojo F, et al. Increased tumour necrosis factor alpha production in mesenteric lymph nodes of cirrhotic patients with ascites. Gut 2003;52:1054-9.
11. Lee FY, Lu RH, Tsai YT, et al. Plasma interleukin-6 levels in patients with cirrhosis. Relationship to endotoxemia, tumor necrosis factor-alpha, and hyperdynamic circulation. Scand J Gastroenterol 1996;31:500-5.
12. Lin RS, Lee FY, Lee SD, et al. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. J Hepatol 1995;22:165-72.
13. Kemp W, Colman J, Thompson K, et al. Norfloxacin treatment for clinically significant portal hypertension: results of a randomised double-blind placebo-controlled crossover trial. Liver Int 2009;29:427-33.
14. Rasaratnam B, Kaye D, Jennings G, et al. The effect of selective intestinal decontamination on the hyperdynamic circulatory state in cirrhosis. A randomized trial. Ann Intern Med 2003;139:186-93.
15. Scarpignato C, Pelosini I. Rifaximin, a poorly absorbed antibiotic: pharmacology and clinical potential. Chemotherapy 2005;51 Suppl 1:36-66.
16. Jiang ZD, DuPont HL. Rifaximin: in vitro and in vivo antibacterial activity--a review. Chemotherapy 2005;51 Suppl 1:67-72.
17. Bass NM, Mullen KD, Sanyal A, et al. Rifaximin treatment in hepatic encephalopathy. N Engl J Med 2010;362:1071-81.
18. Kimer N, Krag A, Moller S, et al. Systematic review with meta-analysis: the effects of rifaximin in hepatic encephalopathy. Aliment Pharmacol Ther 2014;40:123-32.
19. 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;10:815-8.
20. Kalambokis GN, Tsianos EV. Rifaximin reduces endotoxemia and improves liver function and disease severity in patients with decompensated cirrhosis. Hepatology 2012;55:655-6.
21. Bajaj JS, Heuman DM, Sanyal AJ, et al. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. PLoS One 2013;8:e60042.
22. Xu D, Gao J, Gillilland M, 3rd, et al. Rifaximin alters intestinal bacteria and prevents stress-induced gut inflammation and visceral hyperalgesia in rats. Gastroenterology 2014;146:484-96 e4.
23. Kimer N, Pedersen JS, Busk TM, et al. Rifaximin has no effect on hemodynamics in decompensated cirrhosis: A randomized, double-blind, placebo-controlled trial. Hepatology 2017;65:592-603.
24. Systems RD. Product information. Volume 2016, 2016.
25. Duner KI. A new kinetic single-stage Limulus amoebocyte lysate method for the detection of endotoxin in water and plasma. J Biochem Biophys Methods 1993;26:131-42.
26. Mortensen C, Jensen JS, Hobolth L, et al. Association of markers of bacterial translocation with immune activation in decompensated cirrhosis. Eur J Gastroenterol Hepatol 2014;26:1360-6.
27. Lluch J, Servant F, Paisse S, et al. The Characterization of Novel Tissue Microbiota Using an Optimized 16S Metagenomic Sequencing Pipeline. PLoS One 2015;10:e0142334.

28. Paisse S, Valle C, Servant F, et al. Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing. Transfusion 2016.
29. Mortensen MS, Brejnrod AD, Roggenbuck M, et al. The developing hypopharyngeal microbiota in early life. Microbiome 2016;4:70.
30. Hansen CH, Krych L, Nielsen DS, et al. Early life treatment with vancomycin propagates Akkermansia muciniphila and reduces diabetes incidence in the NOD mouse. Diabetologia 2012;55:2285-94.
31. Nunes I, Jacquiod S, Brejnrod A, et al. Coping with copper: legacy effect of copper on potential activity of soil bacteria following a century of exposure. FEMS Microbiol Ecol 2016;92.
32. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 2010;26:2460-1.
33. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 2013;10:996-8.
34. Haas BJ, Chin M, Nusbaum C, et al. How deep is deep enough for RNA-Seq profiling of bacterial transcriptomes? BMC Genomics 2012;13:734.
35. Caporaso JG, Bittinger K, Bushman FD, et al. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 2010;26:266-7.
36. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7:335-6.
37. Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol 2009;26:1641-50.
38. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome Biol 2011;12:R60.
39. Vlachogiannakos J, Daikos G, Thalheimer U, et al. Is bacterial DNA a better marker than endotoxin of bacterial translocation in decompensated cirrhosis? Hepatology 2011;53:2140-1; author reply 41-2.
40. Zapater P, Frances R, Gonzalez-Navajas JM, et al. Serum and ascitic fluid bacterial DNA: a new independent prognostic factor in noninfected patients with cirrhosis. Hepatology 2008;48:1924-31.
41. Bruns T, Reuken PA, Stengel S, et al. The Prognostic Significance Of Bacterial DNA In Patients With Decompensated Cirrhosis and Suspected Infection. Liver Int 2016.
42. Bajaj JS, Heuman DM, Wade JB, et al. Rifaximin improves driving simulator performance in a randomized trial of patients with minimal hepatic encephalopathy. Gastroenterology 2011;140:478-487 e1.
43. Bernardi M, Moreau R, Angeli P, et al. Mechanisms of decompensation and organ failure in cirrhosis: From peripheral arterial vasodilation to systemic inflammation hypothesis. J Hepatol 2015;63:1272-84.
44. Claria J, Stauber RE, Coenraad MJ, et al. Systemic inflammation in decompensated cirrhosis: Characterization and role in acute-on-chronic liver failure. Hepatology 2016;64:1249-64.
45. Lelouvier B, Servant F, Paisse S, et al. Changes in blood microbiota profiles associated with liver fibrosis in obese patients: A pilot analysis. Hepatology 2016;64:2015-2027.
46. Bajaj JS. Review article: potential mechanisms of action of rifaximin in the management of hepatic encephalopathy and other complications of cirrhosis. Aliment Pharmacol Ther 2016;43 Suppl 1:11-26.

47. Koren O, Spor A, Felin J, et al. Human oral, gut, and plaque microbiota in patients with atherosclerosis. Proc Natl Acad Sci U S A 2011;108 Suppl 1:4592-8.
48. Such J, Frances R, Munoz C, et al. Detection and identification of bacterial DNA in patients with cirrhosis and culture-negative, nonneutrocytic ascites. Hepatology 2002;36:135-41.
49. Mehta G, Mookerjee RP, Sharma V, et al. Systemic inflammation is associated with increased intrahepatic resistance and mortality in alcohol-related acute-on-chronic liver failure. Liver Int 2015;35:724-34.
50. Mookerjee RP, Pavesi M, Thomsen KL, et al. Treatment with non-selective beta blockers is associated with reduced severity of systemic inflammation and improved survival of patients with acute-on-chronic liver failure. J Hepatol 2016;64:574-82.





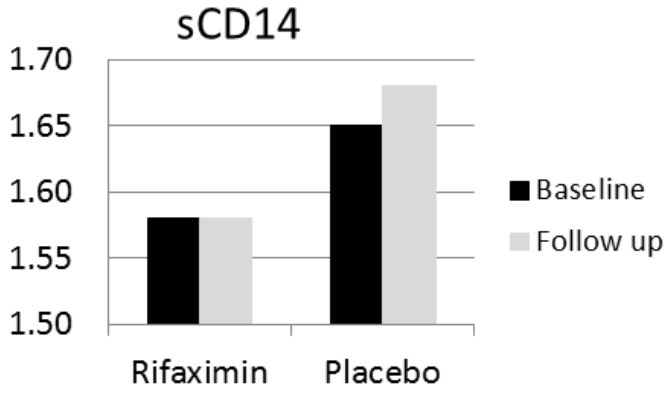


Figure 1: Change in LBP, LPS and sCD14 levels in rifaximin and placebo groups.

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.

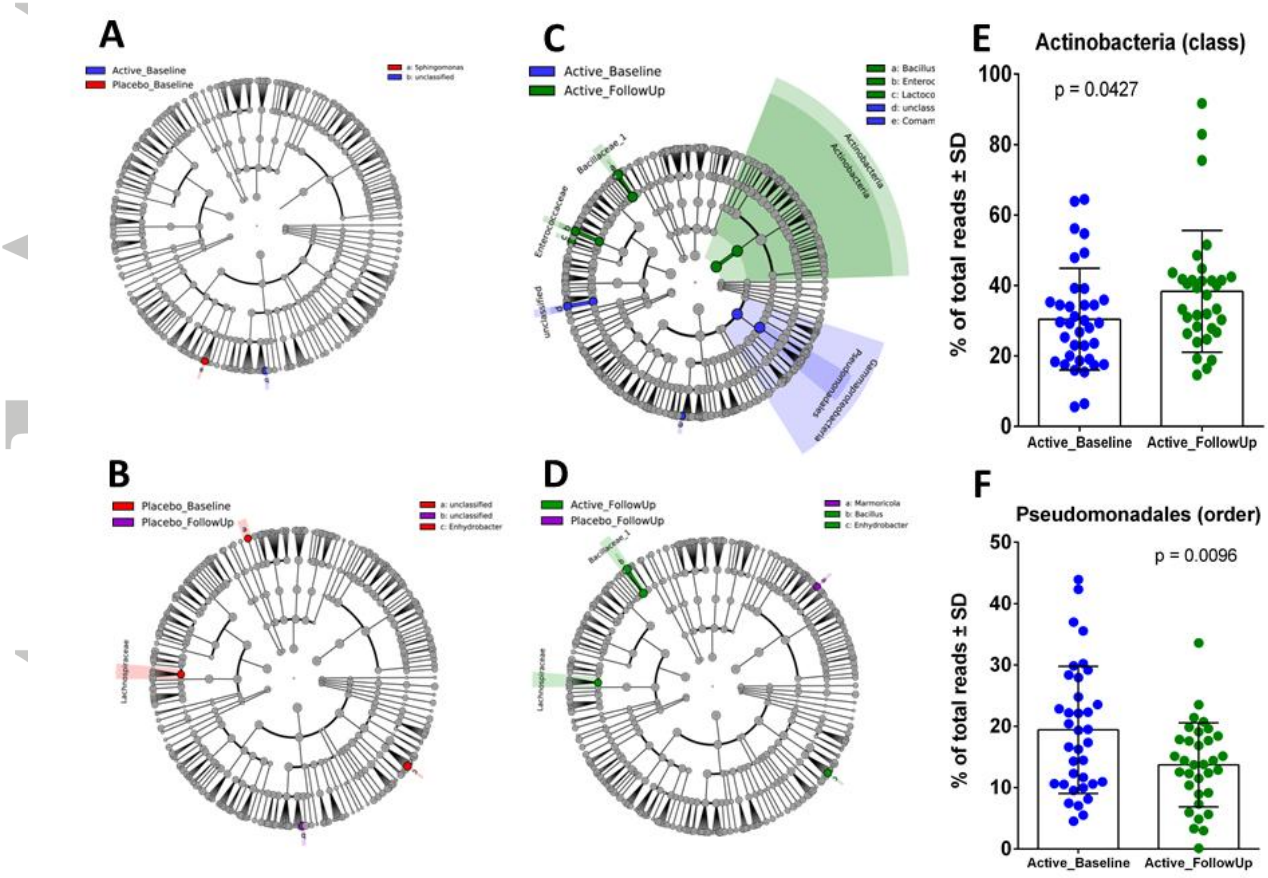


Figure 2: Differential abundances of bacterial taxa in whole blood.

Cladograms derived from pairwise group LEfSe analysis of 16S rRNA gene sequences from whole blood: A) Active Baseline vs Placebo Baseline, B) Placebo Baseline vs Placebo Follow-Up, C) Active Baseline vs Active Follow-Up, and D) Active Follow-Up vs Placebo Follow-Up. 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) Pseudomonales (order) of Active Baseline vs Active Follow-Up.

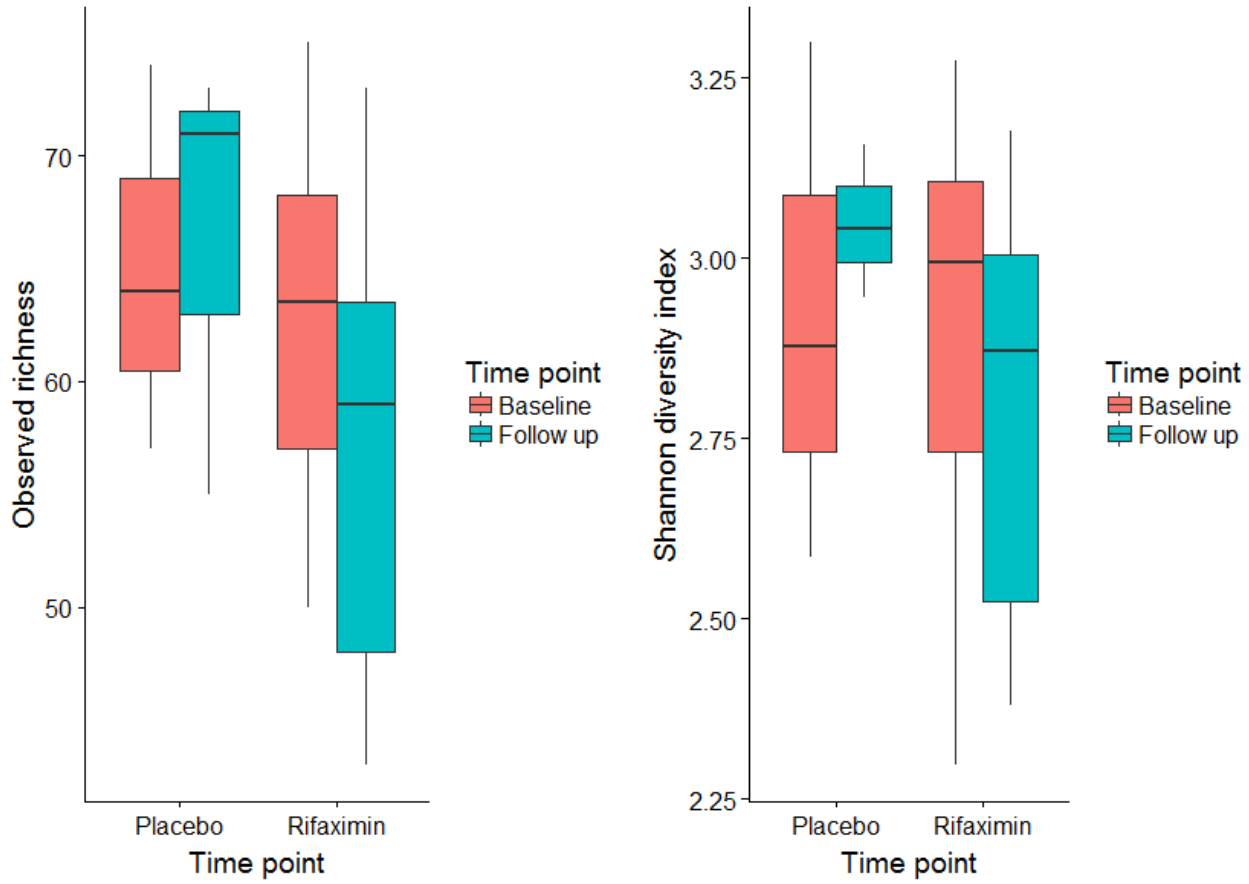


Figure 3: Observed richness of bacteriae in gut flora.

TABLES

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			
Haemoglobin 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 x 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 < -4.

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

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

	Rifaximin (n=36)		Placebo (n=18)		p-value	Normal range[A]	LQL[B]
Inflammation markers							
	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[C]	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[D]
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[D]	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[E]	0.187 (0.236)	0.155 (0.218)	0.255 (0.149)	0.248 (0.219)	0.61	0.0-3.9	3.9[D]
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

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: TNFα: tumour necrosis factor alpha. IL-6: interleukin 6. IL-10: interleukin 10. Hs-CRP: high-sensitivity C-reactive protein. Markers that reflects activation of inflammatory cells and may affect stellate cells in the liver: IL-4: interleukin 4. IL-18: interleukin 18. SDF-1α: Stromal cell-derived factor 1-alpha. TGF-β1: transforming growth factor beta 1. IP10: interferon gamma induced protein 10 (correlated to formation of liver cirrhosis). IL1β: interleukin 1 beta (related to acute inflammation).

A 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.

B LQL: Lowest quantifiable limit, standard test in 40 assays.

C IL-4 was below detection limit in 53 patients (35 rifaximin group/ 18 placebo group) (101 samples).

D IL-10 was below detection limit in 22 patients (18 rifaximin group/ 4 placebo group) (36 samples)

E IL1β was below detection limit in 1 patient (rifaximin group) (2 samples).