HEPATOLOGY

HEPATOLOGY, VOL. 65, NO. 2, 2017

# Rifaximin Has No Effect on Hemodynamics in Decompensated Cirrhosis: A Randomized, Double-Blind, Placebo-Controlled Trial

Nina Kimer,[1,2] Julie Steen Pedersen,[1] Troels Malte Busk,[2] Lise Lotte Gluud,[1] Lise Hobolth,[1,3] Aleksander Krag,[4] Søren Møller,[2] and Flemming Bendtsen,[1] Copenhagen Rifaximin (CoRif) Study Group

**Decompensated cirrhosis is characterized by disturbed systemic and splanchnic hemodynamics. Bacterial translocation from the gut is considered the key driver in this process. Intestinal decontamination with rifaximin may improve hemodynamics. This double-blind, randomized, controlled trial (clinicaltrials.gov, NCT01769040) investigates the effects of rifaximin on hemodynamics, renal function, and vasoactive hormones. We randomized 54 stable outpatients with cirrhosis and ascites to rifaximin 550 mg twice a day (n = 36) or placebo twice a day (n = 18). Forty-five patients were male, mean age 56 years (±8.4), average Child score 8.3 (±1.3), and Model for End-Stage Liver Disease score 11.7 (±3.9). Measurements of hepatic venous pressure gradient, cardiac output, and systemic vascular resistance were made at baseline and after 4 weeks. The glomerular filtration rate and plasma renin, noradrenaline, lipopolysaccharide binding protein, troponin T, and brain natriuretic peptide levels were measured. Rifaximin had no effect on hepatic venous pressure gradient, mean 16.8 ± 3.8 mm Hg at baseline versus 16.6 ± 5.3 mm Hg at follow-up, compared to the placebo, mean 16.4 ± 4 mm Hg at baseline versus 16.3 ± 4.4 mm Hg at follow-up, $P = 0.94$. No effect was found on cardiac output, mean 6.9 ± 1.7 L/min at baseline versus 6.9 ± 2.3 L/min at follow-up, compared to placebo, mean 6.6 ± 1.9 L/min at baseline compared to 6.5 ±2.1 L/min at follow-up, $P = 0.66$. No effects on the glomerular filtration rate, $P = 0.14$, or vasoactive hormones were found. Subgroup analyses on patients with increased lipopolysaccharide binding protein and systemic vascular resistance below the mean (1,011 dynes × s/cm$^5$) revealed no effect of rifaximin. *Conclusion:* Four weeks of treatment with rifaximin did not reduce the hepatic venous pressure gradient or improve systemic hemodynamics in patients with cirrhosis and ascites; rifaximin did not affect glomerular filtration rate or levels of vasoactive hormones. (HEPATOLOGY 2017;65:592-603).**

Portal hypertension (PH) in cirrhosis is driven by two factors: a structural component, characterized by increased intrahepatic resistance due to the disruption of the vascular architecture and by infiltration of fibrosis in the liver parenchyma[(1)]; the second component is dynamic and characterized by endothelial dysfunction in the sinusoidal endothelium and by abnormal contractile properties of the hepatic stellate cells, which are negatively affected by elevated levels of nitric oxides and endotoxins.[(2,3)] Portal pressure is further aggravated by an increased splanchnic inflow due to vasodilatation. Secondarily, systemic vasodilatation develops, leading to a low central blood volume (CBV) and a further reduction in systemic vascular resistance (SVR), which leads to increased cardiac output (CO) and the characteristic hyperdynamic circulation.[(4-7)] Patients with decompensated cirrhosis have increased inflammatory activity, which is hypothesized to be due to bacterial translocation from the gut.[(8-10)] The increased levels of circulating endotoxins and inflammatory cytokines are believed to have a major negative impact on the hyperdynamic circulation.[(11,12)]

*Abbreviations: CBV, central blood volume; CO, cardiac output; GCP, Good Clinical Practice Unit; GFR, glomerular filtration rate; HE, hepatic encephalopathy; HVPG, hepatic venous pressure gradient; LBP, lipopolysaccharide binding protein; LVC, liver vein catheterization; MELD, Model for End-Stage Liver Disease; MHE, minimal HE; PH, portal hypertension; PHES, portosystemic HE score; SVR, systemic vascular resistance.*

*Received April 19, 2016; accepted October 12, 2016.*
*Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.28898/suppinfo.*

Impaired intestinal motility, bacterial overgrowth, and immune dysfunction in the gut are evident in the patient with cirrhosis.[13,14] In controlled trials, intestinal decontamination with norfloxacin has been shown to be beneficial for inflammation, splanchnic hemodynamics, and clinical outcomes in patients with decompensated cirrhosis.[12,15-17]

Rifaximin-α (XIFAXAN; Norgine Denmark A/S, for Alfa Wassermann, Bologna, Italy) is a nonabsorbable antibiotic exerting a broad range of antimicrobial activity, altering the overall composition of the gut microbiota by favoring nonpathogenic bacterial species.[18-21] Rifaximin is used for the prevention of hepatic encephalopathy (HE) and may also have beneficial effects on overt HE and survival.[22] Previous uncontrolled studies have suggested that rifaximin attenuates PH and improves systemic hemodynamics by modulating the levels of endotoxins and nitric oxides arising from the bacterial translocation in patients with decompensated cirrhosis.[23,24] A case–control trial suggested a prolonged effect of rifaximin on the risk of complications to PH.[25] It has also been hypothesized that patients with cirrhosis may benefit from rifaximin as an add-on to nonselective beta-blockers in the treatment of PH.[26] The effects of rifaximin on splanchnic and systemic hemodynamics in decompensated cirrhosis have, to our knowledge, not been assessed in a randomized trial.

The aim of this randomized, double-blind, placebo-controlled trial was to evaluate the effects of rifaximin on splanchnic and systemic hemodynamics and renal function in patients with cirrhosis and ascites.

# Patients and Methods

This double-blind, randomized, and placebo-controlled trial was initiated by the investigators and conducted at a single tertiary center, with patient referrals from six hospitals in the Capital Region and Region Zealand of Denmark. Patients were enrolled between February 2013 and December 2015. The trial was approved by the Danish Health Authorities and the European Medicines Agency (EudraCT 2012-002890-71) and registered on clinicaltrials.gov (NCT01769040). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and the Scientific Ethics Committee of the Capital Region of Denmark (H-1-2012-078). The Good Clinical Practice Unit (GCP) of Copenhagen University Hospital served as the external monitor of the trial.

*Supported by The Research Foundation of The Capital Region of Denmark, Novo Nordisk Foundation (NNF13OC006561), Aase & Ejnar Danielsens Foundation, and Amager and Hvidovre Hospital Research Foundation.*

*N.K. and F.B. received the study medication and placebo free of charge from Norgine Denmark A/S for the present trial (Study code: IIS2011DK01). Norgine Denmark A/S was not involved in the trial design, the conduct of the trial, or the analysis of the trial data.*

*Members of the Copenhagen Rifaximin (CoRif) study group: F. Tofteng, K. Grønbæk, C. Nøjgaard, T. Blixt, I. Vind, M.B. Nielsen, S. Novovic (Gastro Unit, Medical Division, Copenhagen University Hospital Hvidovre, Denmark); E.G. Theisen (Department of Hepatology, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark); L.G. Madsen, S. Dam-Larsen (Department of Internal Medicine, Zealand University Hospital Køge, Denmark); S. Sloth (Gastro Unit, Copenhagen University Hospital, Herlev, Denmark); M. Vester-Andersen (Department of Gastroenterology and Hepatology, Copenhagen University Hospital, Bispebjerg, Denmark); and C.O. Mortensen (Department of Internal Medicine, Copenhagen University Hospital Gentofte, Denmark).*



*View this article online at wileyonlinelibrary.com.*

*DOI 10.1002/hep.28898*

*Potential conflict of interest: Dr. Bendtsen received grants from Norgine. Dr. Krag advises, is on the speakers' bureau, and received grants from Norgine.*

## ARTICLE INFORMATION:

**From the [1]Gastro Unit, Medical Division, and [2]Centre of Functional Imaging and Research, Department of Clinical Physiology and Nuclear Medicine, Copenhagen University Hospital, Hvidovre, Denmark; [3]Department of Gastroenterology and Hepatology, Copenhagen University Hospital, Bispebjerg, Denmark; [4]Department of Gastroenterology and Hepatology, Odense University Hospital, and Institute of Clinical Research, University of Southern Denmark, Odense, Denmark.**

## ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

**Nina Kimer, M.D., Ph.D.**
**Gastro Unit, Medical Division, 360**
**Copenhagen University Hospital Hvidovre**
**Kettegaard Alle 30**
**2650 Hvidovre, Denmark**
**E-mail: Nina.kimer@regionh.dk**
**Tel: +45 38 62 31 81**

The trial protocol is available as Supporting Information.

## OUTCOME MEASURES

The primary outcome measures were hepatic venous pressure gradient (HVPG), CO, and glomerular filtration rate (GFR). Secondary outcomes were mean arterial pressure, SVR, CBV, vasoactive biochemical markers, and reversal of minimal HE (MHE).

## INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria were diagnosis of cirrhosis verified by clinical, biochemical, and ultrasound findings; presence of clinical or ultrasound-verified ascites within the last 3 months; age between 18 and 80 years; and PH with an HVPG of 10 mm Hg or higher. Exclusion criteria were cardiac or respiratory failure, invasive cancer within the past 5 years, clinical or biochemical signs of infection, antibiotic treatment 14 days prior to inclusion, overt HE defined as HE grade 2-4 according to the West Haven criteria, kidney failure with serum creatinine above 200 $\mu$mol/L, transfusion-requiring bleeding within 1 week prior to inclusion, blood hemoglobin level below 5.5 mmol/L, continuous abuse of alcohol with symptoms of withdrawal, or expected survival of less than 3 months. All patients gave informed consent.

## STUDY DESIGN

Patients were randomized in a 2:1 fashion using a computer-generated logarithm provided by our external data manager. Patients were identified by a single four-digit randomization number. All patients and personnel were blinded to the treatment. Study medication was packed according to the randomization list (supplied by the data manager) by the Hospital Pharmacy of the Capital Region of Denmark. The rifaximin group (n = 36) received one tablet of 550 mg twice a day, and the placebo group (n = 18) received one tablet of placebo twice a day. Rifaximin and placebo tablets were similar in color, size, shape, and packing. Decoding could only be performed by the sponsor and principal investigator in unison. Unblinding was performed 6 weeks after the last patient–last visit, when all data were registered in the database and the trial was closed by the GCP unit of Copenhagen University Hospital. The investigational program was performed prior to randomization and after 4 weeks of treatment. All authors had access to the study data and reviewed and approved the final manuscript.

## METHODS

Clinical history data, demographic data on the etiology and complications to cirrhosis, and comorbidities as well as standard biochemistry data were collected. The assessments included a glucose breath test, assessment of the GFR, and a liver vein catheterization (LVC). On the day of inclusion, patients were screened for MHE with the continuous reaction time test, and the psychometric hepatic encephalopathy score (PHES)[27,28]; and standard lab tests and clinical history were recorded. The glucose breath test was performed in the initial 28 patients. After an overnight fast, including abstinence from tobacco and wholegrain products, patients were asked to drink 25 g of glucose diluted in water. Measures of hydrogen in the expiration were recorded, as described.[29]

LVC was performed on the second day, in the morning after an overnight fast. In patients with tense ascites, paracentesis was performed the day before LVC. Guided by fluoroscopy, a balloon catheter was brought through the femoral vein to the hepatic venous system. HVPG was measured as a mean of repeated measurements in at least three different positions. CO was assessed by the indicator dilution technique, using 150 KBq of $^{125}$I-labeled human serum albumin.[6,30,31] Right atrial pressure was measured in the same setting. SVR was calculated as $SVR = 80 \times \frac{Mean\ arterial\ pressure - Right\ atrial\ pressure}{CO}$. Plasma volume and CBV were derived from CO as described.[32]

GFR was measured as plasma clearance by multiple sampling. One injection of 10 mL of isotonic saline with 4 MBq of the indicator $^{51}$chrome-ethylene diamine tetraacetic acid was given readily after the LVC, while the patient was resting in bed.[33] A gamma-counter was used to assess the radioactivity of $^{51}$chrome-ethylene diamine tetraacetic acid (Perkin-Elmer, New York, NY) in the blood, and clearance was assessed as the plasma concentration time curve and the area under the plasma indicator curve. Plasma clearance was derived from the ratio between the injected amount of indicator and the area under the indicator curve.[33,34]

Before infusing radioactive isotopes, 25 mL of blood was procured from the femoral artery for the analyses of arterial ammonia, renin, and vasoactive markers; all tubes were placed on ice immediately after sampling.

Renin levels were estimated with a commercially available immunoradiometric assay (DGR International, Inc., Hamburg Germany). The detection limit was 0.31 g/mL, and the intra-assay and interassay variation was 2%.

Brain natriuretic peptide and troponin T concentrations were measured using the standard biochemical test by ElektroChemiLuminescens (Roche Diagnostics A/S).

Noradrenaline and adrenaline were measured as the stable metabolites methoxy-noradrenaline and methoxy-adrenaline using a commercially available kit (Acquity UPLC IClass Xevo TQ-S; Waters Corporation, Milford, MA) with a lower detection limit of 0.1 nmol/L.

Lipopolysaccharide binding protein (LBP) was measured in ethylene diamine tetraacetic acid plasma with a commercially available, solid-phase enzyme-linked immunosorbent assay (Vaiomer SAS, Toulouse, France). Protease activity was assessed by recording absorbance at 405 nm. The minimal detection limit was 3.5 $\mu$g/mL.

Patients were seen in the outpatient clinic after 14 days of treatment, where complications and side effects were registered, together with counting the residual tablets and handing out medicine for the next 14 days of treatment. After 4 weeks, patients were readmitted to the hospital for repetition of the investigational program.

## STATISTICAL ANALYSIS

For sample size calculation, a type I error of 0.05 was chosen. Sample size calculations were performed for the primary outcomes HVPG and GFR (a mean HVPG of 18 mm Hg and a mean GFR of 69 mL/minute).[35,36] Sample size calculation for CO was not performed. In a paired design, 48 patients completing the trial protocol were required to detect a 20% reduction in HVPG or a 20% increase in GFR, which we estimated were the smallest clinically relevant changes. With an expected 20% dropout rate, we planned to include 57 patients.

Intention-to-treat and per-protocol statistical analyses were planned. Differences between the two groups were assessed using the unpaired *t* testing of delta values (the difference between follow-up and baseline). Intestinal decontamination has been suggested to be more beneficial in patients with systemic vasodilation,[12] and we therefore performed *post hoc* analyses in the following subgroups: patients with low SVR, presence of MHE, activated LBP, or treatment with beta-blockers. Analyses of covariance (repeated measures), with Model for End-Stage Liver Disease (MELD) score, Child score, severity of ascites, white blood cells, SVR, PHES as a measure of MHE, and LBP as covariates, were performed. Data were handled using SAS statistical software (v9.4 and Enterprise v7.1; SAS Institute, Cary, NC) and GraphPad (Prism 6.0.7).

# Results

## PATIENTS

A total of 295 patients were assessed for eligibility (Fig. 1). Fifty-four patients (45 men, 9 women) fulfilled the inclusion criteria and were randomized. Inclusion into the study was stopped in accordance with International Conference for Harmonization–GCP guidelines when 49 patients had completed the trial protocol; thus, the sample size was reached.[37] The majority of patients (94%) had ascites at the time of inclusion. At the time of screening (2-10 weeks prior), all patients had ascites, which was verified using puncture or ultrasound. Thirty-nine patients had tense or moderate ascites. Due to adjustments in diuretics, 3 patients had remitted their ascites; and another 6 had mobilized ascites that were only visible on ultrasound at the time of inclusion. Ten patients had refractory ascites. Six patients had tense ascites at both inclusion and follow-up and were relieved with paracentesis prior to LVC. Four patients were relieved with paracentesis within 1 week prior to inclusion. Forty-nine patients received diuretics in varying doses. Three patients did not tolerate diuretics due to previous episodes of hepatorenal syndrome, and 2 patients had stopped taking diuretics due to the side effects. All patients were kept on a stable dose of diuretics 1 week prior to and during the trial. The median age of the participants was 56 years (range 33-74), the median MELD score was 11 (range 6-25), and the median Child score was 8 (range 7-12). Alcohol was the predominant etiology (78% of patients). Ten patients had had an episode of overt HE prior to trial inclusion; 11 patients had bled from esophageal varices. Fifteen patients (10 rifaximin/5 placebo) were treated with concomitant beta-blockers (Table 1). No hemodynamic or biochemical differences were detected between the two groups; patients in the rifaximin group had a higher MELD score, which probably reflects the higher number of Child C

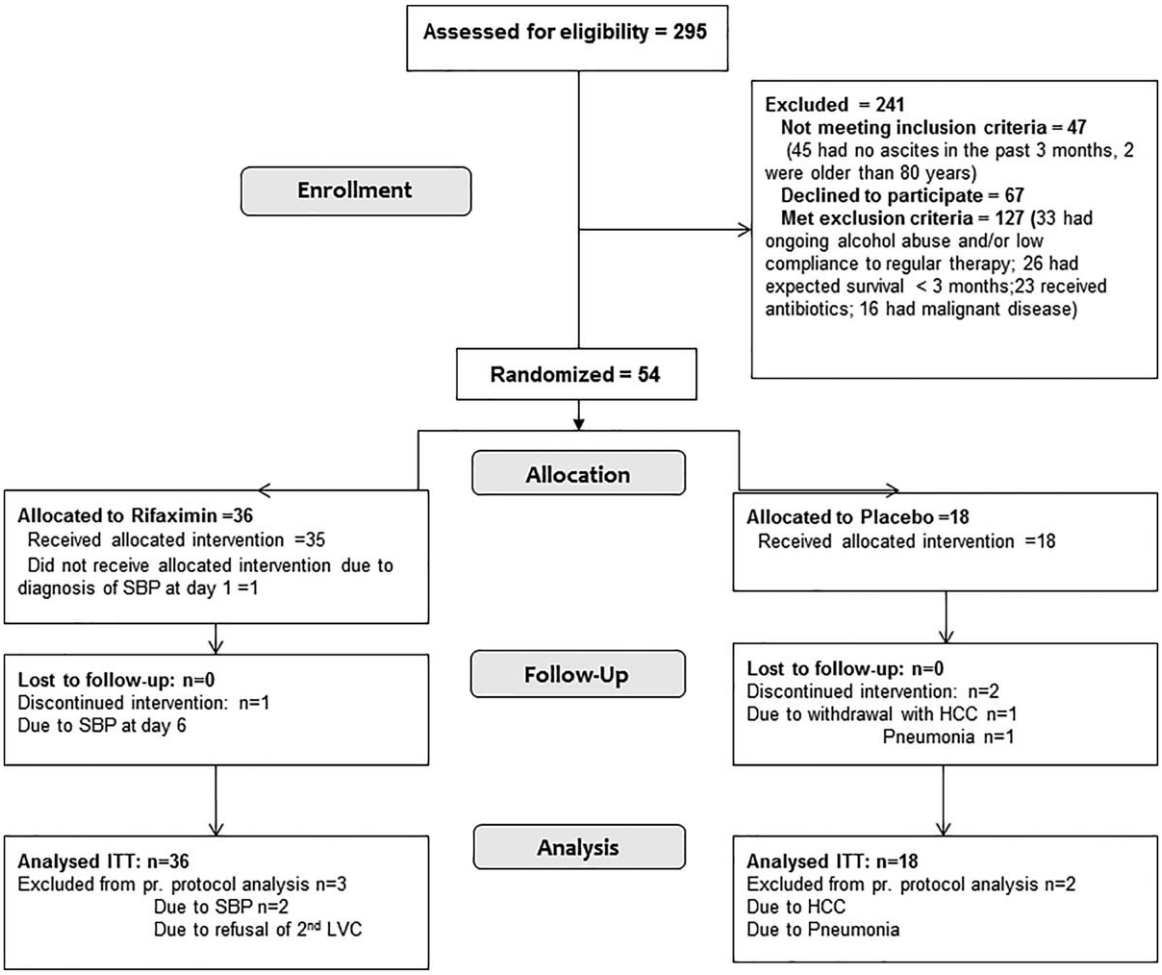


**FIG. 1.** The CONSORT trial flow chart of patients. Abbreviations: HCC, hepatocellular carcinoma; SBP, spontaneous bacterial peritonitis.

patients randomized to this group. Compliance with study treatment was 97.7%.

The first 28 patients who were included were assessed for small intestinal bacterial overgrowth with the glucose breath test. No patients had small intestinal bacterial overgrowth, and two of the 28 tests were invalid, most likely due to a lack of fasting and abstinence from tobacco before testing. Due to continuous negative testing, the assessment was abandoned as recommended by GCP guidelines.[37]

## PRIMARY ENDPOINTS

### Hepatic and Systemic Hemodynamics

Rifaximin had no effect on HVPG (mean and standard deviation 16.8 ± 3.8 mm Hg at baseline versus 16.6 ± 5.3 mm Hg at follow-up) compared to placebo (16.4 ± 4 mm Hg at baseline versus 16.3 ± 4.4 mm Hg at follow-up), $P = 0.94$ (Fig. 2). Similarly, no effect could be demonstrated on systemic hemodynamics as reflected by the CO, SVR (Fig. 2), plasma volume, and CBV (Table 2; Supporting Fig. S2). Heart rate decreased in the rifaximin group (76 ± 13 bpm at baseline versus 73 ± 13 bpm at follow-up) compared to an increase in the placebo group (76 ± 15 bpm at baseline versus 79 ± 15 bpm at follow-up), $P = 0.034$. A subgroup analysis of patients with SVR below the mean (1011 dynes × s/cm$^5$, rifaximin n = 24, placebo n = 10) and patients treated with beta-blockers (rifaximin n = 10, placebo n = 5) did not reveal any effect of rifaximin on patients with disturbed vascular resistance.

**TABLE 1. Baseline Patient Characteristics**

| | Rifaximin (n = 36) | Placebo (n = 18) |
|---|---|---|
| Age | 58.5 (33-68) | 52.5 (34-74) |
| Sex (male/female) | 31/5 | 14/4 |
| Etiology | | |
| Alcohol | 29 | 13 |
| Nonalcoholic steatohepatitis | 1 | 1 |
| Alcohol/hepatitis C | 1 | 2 |
| Hepatitis B | 1 | |
| Alcohol/hepatitis B | 1 | |
| Hepatitis C/hepatitis B | 1 | |
| Autoimmune | 1 | |
| Alpha$_1$-antitrypsin/alcohol | | 1 |
| Cryptogenic | 1 | 1 |
| Child class B/C | 27/9 | 17/1 |
| MELD | 12 (6-25) | 9.5 (6-15) |
| Complications to cirrhosis, n (%) | | |
| Previous HE | 8 (22%) | 2 (11%) |
| Varices grade 1/2/3, n | 13/12/4 | 5/2/2 |
| Previous variceal bleeding | 8 (22%) | 3 (17%) |
| Beta-blocker therapy, n (%) | 10 (28%) | 5 (28%) |
| Dose | 80 mg (10-120)*,† | 80 mg (5-100)* |
| Furosemide, n (%) | 20 (56%) | 14 (78%) |
| Dose | 60 mg (30-160) | 60 mg (40-160) |
| Spironolactone, n (%) | 30 (83%) | 15 (83%) |
| Dose | 100 mg (25-300) | 100 mg (25-300) |
| Ascites (mild/moderate/tense) | 13/14/7 | 8/6/3 |
| Biochemistry | | |
| Albumin (g/L) | 28.5 (21-40) | 32 (24-43) |
| Coagulation factor (II, VII, X) | 0.505 (0.26-1.21) | 0.565 (0.36-0.96) |
| Bilirubin ($\mu$mol/L) | 24 (8-166) | 16.5 (5-40) |
| Creatinine ($\mu$mol/L) | 60 (43-171) | 73 (44-146) |
| Platelets ($10^9$/L) | 131 (27-562) | 151.5 (56-275) |
| Hemoglobin (mmol/L) | 7.6 (5.3-9.6) | 7.85 (5-9.8) |
| White blood cells ($10^9$/L) | 6.25 (2.6-13.2) | 7.05 (3.6-16.9) |
| Alanine transaminase (U/L) | 25.5 (10-153) | 28 (15-56) |
| Alkaline phosphatase (U/L) | 121.5 (53-1,200) | 146 (47-459) |
| C-reactive protein (mg/L) | 5 (0.3-40) | 5 (0-31) |
| Continuous reaction time index | 1.602 (0.466-4.29) | 1.336 (0.759-3.14) |
| PHES | –6 (–13 to 3) | –7 (–15 to 2) |
| GFR mL/hour | 87 (26.4-127.1) | 78.55 (34.4-142.9) |
| Mean arterial pressure (mm Hg) | 86.5 (66-115) | 85 (69-98) |

Values are given as median and minimum to maximum as they do not follow normal distribution, unless otherwise stated.
*One patient received 100 mg metoprololsuccinat daily.
†One patient received 12.5 mg carvedilol daily.

## Kidney Function

Eight patients had signs of kidney failure, with a GFR below 60 mL/min. Twenty-two patients had clinically normal kidney function but decreased GFR between 60 and 90 mL/min. The remaining 24 patients had GFR above 90 mL/min. Renin levels were increased in 35 patients at baseline. The GFR and plasma renin were unaltered by the study treatment (Table 2).

# SECONDARY END POINTS

## Vasoactive Markers

Noradrenaline was unchanged by rifaximin (0.47 ± 0.23 nmol/L at baseline versus 0.47 ± 0.22 at follow-up) compared with the placebo group (0.44 ± 0.24 nmol/L at baseline versus 0.52 ± 0.25 at follow-up), $P = 0.16$ (Table 2). Likewise, no changes were detected in adrenaline. Levels of pro–brain natriuretic peptide and troponin T were elevated in 27 (rifaximin 16, placebo 11) and 21 (rifaximin 13, placebo 8) patients, respectively. No changes were detected in pro–brain natriuretic peptide or troponin T (Table 2).

Levels of LBP significantly decreased in the rifaximin group, $P = 0.018$ (Table 2). LBP was activated to levels above 5.9 $\mu$g/mL in 37 patients (25 rifaximin, 12 placebo). The subgroup analysis on these patients with activated LBP at baseline did not show an effect of rifaximin on the primary outcomes (HVPG, 17 ± 4 mm Hg at baseline versus 16.9 ± 5.5 mm Hg at follow-up in the rifaximin group compared to 16.4 ± 4.2 mm Hg at baseline versus 16.5 ± 4.7 mm Hg at follow-up in the placebo group, $P = 0.86$; CO, 6.6 ± 1.7 L/min at baseline versus 6.8 ± 2.2 L/min at follow-up in the rifaximin group compared to 7.3 ± 1.9 L/min at baseline versus 7.1 ± 2.3 L/min at follow-up in the placebo group, $P = 0.64$; GFR, 85 ± 26 mL/hour at baseline versus 84 ± 32 mL/hour at follow-up in the rifaximin group compared to 94 ± 28 mL/hour at baseline versus 84 ± 21 mL/hour at follow-up in the placebo group, $P = 0.13$). None of the secondary hemodynamic or vasoactive parameters or the MHE and ammonia levels were affected by rifaximin treatment in the subgroup analyses (data not shown).

## Biochemistry

The standard biochemical measures were unaffected by rifaximin treatment (Supporting Table S1).

## Hepatic Encephalopathy

As a measure of MHE, the continuous reaction time test improved significantly in the rifaximin group (1.711 ± 0.74 at baseline versus 2.069 ± 0.69 at

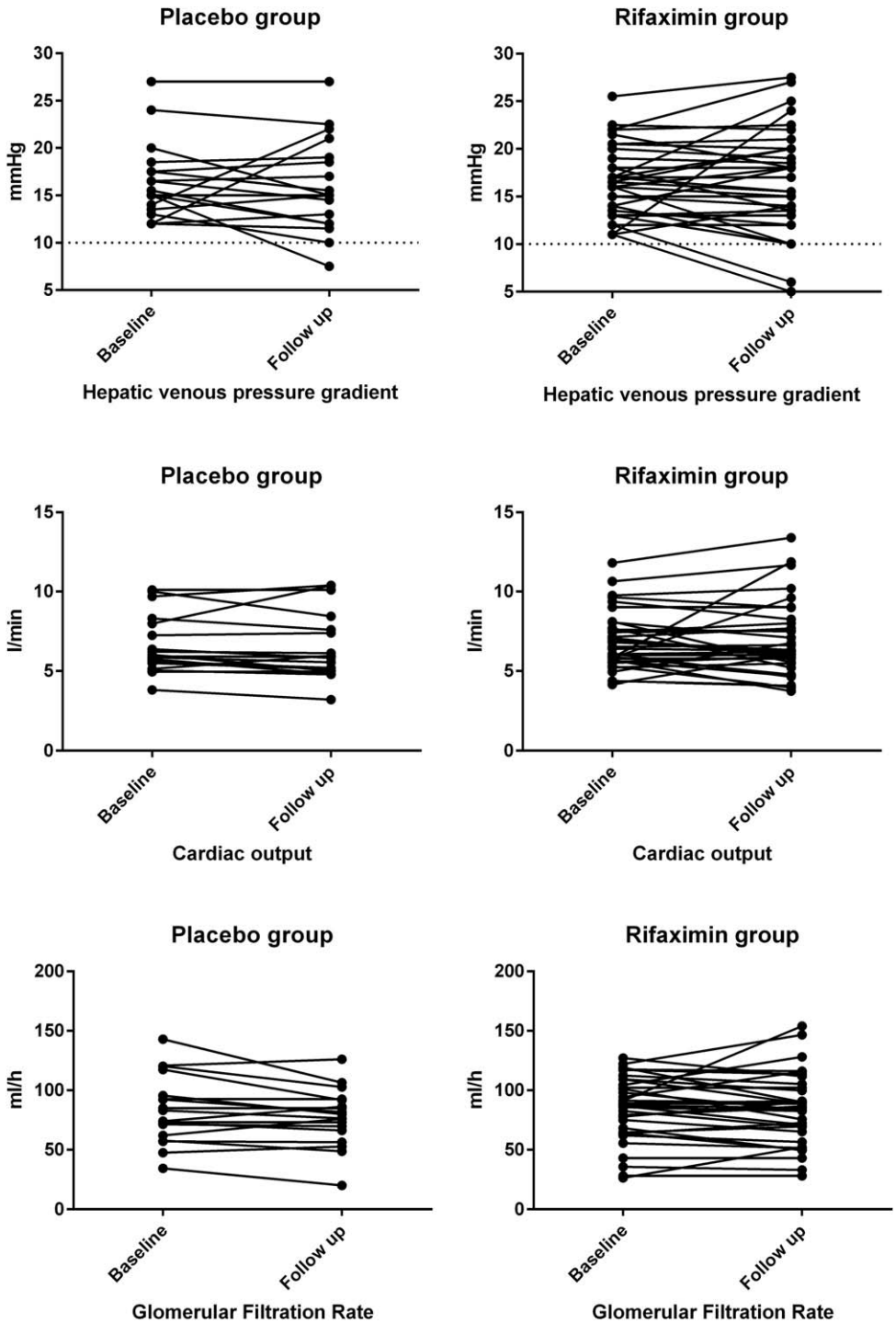


**FIG. 2.** Changes in primary outcomes before and after rifaximin/placebo.

follow-up) compared with the placebo group (1.647 ± 0.7 at baseline versus 1.574 ± 0.53 at follow-up). However, the PHES and arterial ammonia remained indifferent to rifaximin treatment (Table 2). The subgroup analysis of patients with MHE, defined by a PHES below –4 (rifaximin n = 22, placebo n = 12), did not change the results (change in PHES, P = 0.47; continuous reaction time test, $P = 0.053$; and arterial ammonia, P = 0.77). A repeated measures analysis of covariance, which was adjusted for time and

**TABLE 2. Primary and Secondary Outcomes Before and After Treatment**

| | Rifaximin (n = 36) | | | Placebo (n = 18) | | | | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | Follow-up | Mean Delta | Baseline | Follow-up | Mean Delta | $P^{\dagger}$ | Normal Range[‡] |
| Child score | 8.6 (1.3) | 8.1 (1.5) | –0.44 | 7.8 (0.9) | 7.3 (0.9) | –0.56 | 0.59 | NA |
| MELD | 12.5 (4.3) | 12.2 (4) | –0.33 | 9.9 (2.4) | 9.9 (2.2) | –0.056 | 0.30 | NA |
| Splanchnic and systemic hemodynamics | | | | | | | | |
| HVPG* (mm Hg) | 16.8 (3.8) | 16.6 (5.3) | –0.125 | 16.4 (4.0) | 16.3 (4.4) | –0.056 | 0.94 | 0-5 |
| WHVP (mm Hg) | 25.7 (5.4) | 25.6 (6.6) | –0.097 | 24.5 (5.1) | 24.9 (5.8) | 0.417 | 0.65 | NA |
| FHPG (mm Hg) | 9.1 (4) | 9.1 (3.1) | 0.028 | 8.1 (3.1) | 8.6 (2.2) | 0.44 | 0.62 | NA |
| CO* (L/min) | 6.9 (1.7) | 6.9 (2.3) | –0.001 | 6.6 (1.9) | 6.5 (2.1) | –0.151 | 0.66 | 4-8 |
| Mean arterial pressure (mm Hg) | 86 (12.4) | 87 (11.4) | 0.694 | 84 (8.8) | 84 (7.8) | –0.111 | 0.72 | 70-105 |
| Cardiac index (L/min/$m^2$) | 3.7 (0.95) | 3.7 (1.2) | 0.04 | 3.5 (0.82) | 3.4 (0.88) | –0.14 | 0.32 | 2.5-4 |
| Heart rate (bpm) | 76 (12) | 73 (13) | –2.94 | 75.5 (15) | 79 (15) | 3.61 | **0.034** | 50-90 |
| SVR (dyn x s/$cm^5$) | 989 (286) | 1,004 (320) | 15 | 1,054 (334) | 1,137 (433) | 82.83 | 0.15 | <1200 |
| Plasma volume (L) | 4.02 (0.79) | 4.07 (0.95) | 0.049 | 3.89 (0.74) | 3.92 (0.95) | 0.035 | 0.94 | 2.2-3.8 |
| Central blood volume (L) | 1.67 (0.32) | 1.77 (0.7) | 0.098 | 1.59 (0.35) | 1.62 (0.65) | 0.037 | 0.70 | NA |
| Kidney function | | | | | | | | |
| GFR* (mL/hour) | 83.7 (26.8) | 84.7 (29.4) | 0.86 | 83.0 (28.6) | 77.4 (23.8) | –5.57 | 0.14 | >90 |
| Plasma renin (ng/L) | 230.28 (530.63) | 225.92 (550.67) | –4.36 | 111.22 (141.3) | 171.17 (156.3) | 59.94 | 0.12 | 2.8-40.0 |
| Vasoactive markers | | | | | | | | |
| Methoxy-noradrenaline (nmol/L) | 0.47 (0.23) | 0.47 (0.22) | 0.001 | 0.44 (0.24) | 0.52 (0.25) | 0.073 | 0.16 | <1.1 |
| Methoxy-adrenaline (nmol/L) | 0.287 (0.104) | 0.282 (0.093) | –0.004 | 0.275 (0.116) | 0.289 (0.117) | 0.013 | 0.28 | <0.47 |
| Troponin T (ng/L) | 17.44 (14.64) | 17.53 (15.69) | 0.083 | 14.17 (5.37) | 14.0 (5.27) | –0.167 | 0.79 | <15 |
| Brain natriuretic peptide ($\mu$g/L) | 27.14 (50.87) | 26.78 (48.94) | –0.35 | 24.96 (24.35) | 16.5 (17.43) | –8.45 | 0.09 | <14 |
| MHE | | | | | | | | |
| Continuous reaction time Index | 1.711 (0.74) | 2.069 (0.69) | 0.358 | 1.647 (0.7) | 1.574 (0.53) | –0.072 | **0.018** | >1.9 |
| PHES | –5.9 (4.3) | –5.1 (4.3) | 0.82 | –6.9 (5.0) | –6.4 (6.1) | 0.444 | 0.70 | ≥–4 |
| Arterial ammonia ($\mu$mol/L) | 55.6 (17.7) | 62.1 (54.9) | 7.59 | 49.4 (18.6) | 65.7 (42.2) | 16.25 | 0.52 | <60 |
| Bacterial translocation | | | | | | | | |
| LBP ($\mu$g/mL) | 8.09 (4.24) | 7.52 (3.77) | –0.57 | 7.46 (3.46) | 7.99 (3.68) | 0.53 | 0.018 | 0-5 |

*Primary endpoints were HVPG, CO, and GFR.
†*T* testing of delta values. Defined as follow-up minus baseline. All numbers are given in mean (standard deviation).
‡Normal range for healthy individuals. In case of sex-specific normal ranges, values for males are given.
Abbreviations: FHVP, free hepatic venous pressure; WHVP, wedged hepatic venous pressure.

**TABLE 3. Adverse Events**

| | Rifaximin (n = 36) | Placebo Group (n = 18) |
|---|---|---|
| Serious adverse events | 4 | 1 |
| | SBP (2 cases) | Pneumonia |
| | Duodenal ulcer | |
| | Post–LVC infection | |
| Adverse events | 38 | 16 |
| Possibly related to study medication | Mild abdominal pain (6 cases) | Mild abdominal pain |
| | Reflux | Reflux |
| | Nausea (2 cases) | Nausea |
| | Diarrhea (5 cases) | Diarrhea |
| | Obstipation (4 cases) | Hunger |
| | Flatulence | Flatulence |
| | Vomiting (2 cases) | Vomiting |
| Unlikely related to study medication | Back and neck pain (4 cases) | Back pain (2 cases) |
| | Dizziness (4 cases) | Hematoma after LVC (2 cases) |
| | Coughing (2 cases) | Dizziness (2 cases) |
| | Mastalgia (2 cases) | Asymptomatic C. diff. |
| | Eczema on feet | Influenza |
| | Hematochezia | Peripheral edema |
| | Fall | |
| | Chest pain | |
| | Hematuria | |

Abbreviations: C. diff., *Clostridium difficile*; SBP, spontaneous bacterial peritonitis.

group, was performed on all outcomes with the MELD score, the Child score, the severity of ascites, LBP, white blood cells, SVR, and PHES as covariates. The results of the primary outcomes, HVPG, CO, and GFR, are stated in Supporting Table S2. No changes in the overall results were found. All statistical exercises were repeated per protocol analyses. This did not change the results.

## SAFETY

Three patients were withdrawn from the trial at days 1, 6, and 23 due to development of infections (Table 3). One patient was diagnosed with hepatocellular carcinoma at day 18 and was withdrawn thereafter, and 1 patient completed the trial but refused a second LVC. Serious adverse events were registered in 5 cases (2 patients developed spontaneous bacterial peritonitis, 1 patient was diagnosed with duodenal ulcer, and 1 patient developed post–LVC infection in the inguinal region after the second LVC in the rifaximin group; 1 patient developed pneumonia in the placebo group); no patients presented with HE or variceal bleeding; and no patients died during the trial.

# Discussion

The main findings after 4 weeks of treatment with rifaximin in patients with decompensated cirrhosis and ascites were as follows: no change in the HVPG, systemic hemodynamics, or renal function. We also did not find evidence to support an effect on systemic or arteriolar vasodilation. In this exploratory trial, the PHES as well as arterial ammonia were likewise unaffected by rifaximin.

Rifaximin may facilitate an overproduction of bacterial species that generate less oxidative stress, less nitric oxide production, and less amino acid production, which is hypothesized to explain the known beneficial effects of the drug in reducing recurrent HE.[22,38] The present trial investigated the effects of rifaximin on systemic and splanchnic hemodynamics following the current hypothesis that bacterial translocation induces an inflammatory response, which again agitates the dysfunction of the hepatic stellate cells and the hyperdynamic circulation in cirrhosis. Using rifaximin for intestinal decontamination, this cascade of events is anticipated to be disrupted, facilitating an improvement in systemic hemodynamics.[39] This theory has previously been tested in a small uncontrolled study demonstrating a decrease in HVPG and an increase in CO and SVR in all of the 13 included patients after 4 weeks of rifaximin treatment.[23] Further, the interleukin-6 and tumor necrosis factor-α levels were also attenuated by treatment. Another uncontrolled study of 30 patients found a substantial effect of rifaximin on both the HVPG and endotoxin levels.[40] This study was followed by a case–control study, in which the cohort was matched to 46 controls, showing beneficial long-term effects of rifaximin on HVPG,

survival, and complications of cirrhosis.[25] It is worth noting that only the 23 responders to treatment with rifaximin were rolled over into the case-controlled study, which may bias the results in favor of rifaximin. Additionally, the study provides no data on alcohol consumption or comorbidities in either group, facts that may indeed affect complication rates and cirrhosis survival. About half of the patients who were included in these two trials were Child C. There is a high risk of bias in these studies due to the nonrandomized designs, which imply a risk of false-positive results.

One may hypothesize that the possible effect of rifaximin is clearer in the more severely disturbed hemodynamic state; this effect may not be repeatable in a trial with only 20% of Child C patients, such as ours. The subgroup analysis (paired *t* test) on primary outcomes in Child C (9 patients) in the rifaximin group did not reveal a significant treatment effect. The majority of patients included in the present trial (85%) were hemodynamically disturbed, with SVR values below 1,200 dynes × s/cm$^5$ and a mean CO of 6.7 L/minute. The subgroup analysis of patients with SVR values below average (1,011 dynes × s/cm$^5$, n = 34) and of patients with activated LBP (LBP values above 5.9 $\mu$g/mL, n = 37) also did not demonstrate a beneficial effect of rifaximin. The results might differ markedly had we investigated the effects of rifaximin in the unstable patient recovering from acute-on-chronic liver failure or recent infection.

Some considerations about our trial methods need elaboration. Patients were treated for 4 weeks, allowing for a short but stable treatment period and a quick return to the hospital for reassessment. The treatment duration has varied a great deal in rifaximin trials, depending on the study aim, the patient categories, and the methods of assessment.[23,25,41] A longer treatment period may impact HVPG, but to date this has not been investigated.

A period of 3 years was required to enroll 54 stable patients in the present trial, with a screen failure ratio of 1:5.5. It can be argued that the 54 randomized patients were a highly selected group with no evidence of malignancy, acute or recent infection, or alcohol abuse, which are endemic conditions in chronic liver disease. This selection of patients may bias the results. Alcohol use was self-reported, and s-ethanol tests were not performed. An underreporting of alcohol use is possible in this patient group; however, no patients showed signs of active alcoholism or withdrawal symptoms during follow-up. Antibiotics are frequently used in patients with cirrhosis, and though we tried to avoid this, 1 patient received 3 days of penicillin for an asymptomatic urinary tract infection. One patient was infected with *Clostridium difficile*, and another patient tested positive for vancomycin-resistant *Escherichia coli*; both were asymptomatic throughout the study period and received no antibiotics during the 4 weeks. Treatment with beta-blockers may compromise the effects of rifaximin because a recent trial has suggested some effect of beta-blockers on gut microbiota.[42] Fifteen patients received beta-blockers, but the subgroup analysis revealed no difference between these groups. A recently published abstract explored the potential role of rifaximin as an addition to propranolol.[26] Seventeen patients received combination therapy, which led to a higher decrease in HVPG after 3 months of treatment. Rifaximin also reduced the efficient dose of propranolol to a mean of 127 mg/day. Further data from this and other studies may tell us more about the effects of combination therapy.

The strength of the trial is clearly the randomized design, with personnel, patients, and outcome assessors blinded to treatment until the results were ready for analysis. Compliance with medication and investigations was high, and withdrawal was less than expected. Likewise, concomitant medical treatment was stable, with no variation in benzodiazepines, pain relievers, diuretics, or beta-blockers during the study period. Variance between baseline and follow-up was low for virtually all outcomes, suggesting a robust finding, which will not be influenced by longer treatment duration or an increased number of patients.

We have investigated the circulatory parameters related to cirrhosis extensively, addressing both hepatic and cardiac measures of dysfunction. At the same time, we addressed the possible effects of decreasing the inflammatory response on renal and arteriolar function and vasodilation by assessing vasoactive marker concentrations in relation to rifaximin. At the organ level, the neuropsychiatric parameters did not reveal any change in MHE, nor did rifaximin give rise to improvement in kidney function. Overall, these consistent results rather clearly support the conclusion that rifaximin has no benefits on the hemodynamics in decompensated patients without signs of overt HE.

In conclusion, rifaximin is efficient for recurrent HE, and evidence may support its use in acute overt HE or MHE patients.[22,38,43] This study does not support the use of rifaximin outside this indication. Further studies within the area should focus on the pathophysiological mechanisms that form the basis of how rifaximin changes the outcomes for patients with

HE and MHE and should focus on the mechanisms of preventing bacterial translocation from the gut.

*Acknowledgment:* We are indebted to bioanalysts Katrine Lyngby, Bente Pedersen, and their colleagues at the Department of Clinical Physiology and Nuclear Medicine, Center for Diagnostic Imaging and Research, Copenhagen University Hospital, Hvidovre, Denmark, for their invaluable support in the collection and handling of biological material during investigational procedures. A special thanks goes to all members of the study group, who supported us in finding eligible patients.

## REFERENCES

1) Sethasine S, Jain D, Groszmann RJ, Garcia-Tsao G. Quantitative histological–hemodynamic correlations in cirrhosis. Hepatology 2012;55:1146-1153.
2) Iwakiri Y. Endothelial dysfunction in the regulation of cirrhosis and portal hypertension. Liver Int 2012;32:199-213.
3) Iwakiri Y, Groszmann RJ. Vascular endothelial dysfunction in cirrhosis. J Hepatol 2007;46:927-934.
4) Villanueva C, Albillos A, Genesca J, Abraldes JG, Calleja JL, Aracil C, et al. Development of hyperdynamic circulation and response to beta-blockers in compensated cirrhosis with portal hypertension. Hepatology 2016;63:197-206.
5) Iwakiri Y, Groszmann RJ. The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule. Hepatology 2006;43(2 Suppl. 1):S121-S131.
6) Bosch J, Abraldes JG, Berzigotti A, Garcia-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. Nat Rev Gastroenterol Hepatol 2009;6:573-582.
7) 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.
8) Tilg H, Wilmer A, Vogel W, Herold M, Nolchen B, Judmaier G, et al. Serum levels of cytokines in chronic liver diseases. Gastroenterology 1992;103:264-274.
9) Mortensen C, Andersen O, Krag A, Bendtsen F, Moller S. High-sensitivity C-reactive protein levels predict survival and are related to haemodynamics in alcoholic cirrhosis. Eur J Gastroenterol Hepatol 2012;24:619-626.
10) Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. J Hepatol 2014;60:197-209.
11) Lin RS, Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, 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;22:165-172.
12) Albillos A, de la Hera A, Gonzalez M, Moya JL, Calleja JL, Monserrat J, et al. Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. Hepatology 2003;37:208-217.
13) Bellot P, Garcia-Pagan JC, Frances R, Abraldes JG, Navasa M, Perez-Mateo M, et al. Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. Hepatology 2010;52:2044-2052.
14) Cirera I, Bauer TM, Navasa M, Vila J, Grande L, Taura P, et al. Bacterial translocation of enteric organisms in patients with cirrhosis. J Hepatol 2001;34:32-37.
15) Kemp W, Colman J, Thompson K, Madan A, Vincent M, Chin-Dusting J, et al. Norfloxacin treatment for clinically significant portal hypertension: results of a randomised double-blind placebo-controlled crossover trial. Liver Int 2009;29:427-433.
16) Moreau R, Bureau C, Perarnau JM, Thevenot T, Saliba F, Ollivier-Hourmand I, et al. Effects of norfloxacin therapy on survival in patients with Child-Pugh class C cirrhosis: Results of a randomized, double-blind, placebo-controlled, multicenter trial. Hepatology 2015;62:282A.
17) Fernandez J, Navasa M, Planas R, Montoliu S, Monfort D, Soriano G, et al. Primary prophylaxis of spontaneous bacterial peritonitis delays hepatorenal syndrome and improves survival in cirrhosis. Gastroenterology 2007;133:818-824.
18) 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.
19) Bajaj JS, Heuman DM, Sanyal AJ, Hylemon PB, Sterling RK, Stravitz RT, et al. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. PLoS One 2013;8:e60042.
20) Ridlon JM, Alves JM, Hylemon PB, Bajaj JS. Cirrhosis, bile acids and gut microbiota: unraveling a complex relationship. Gut Microbes 2013;4:382-387.
21) Scarpignato C, Pelosini I. Rifaximin, a poorly absorbed antibiotic: pharmacology and clinical potential. Chemotherapy 2005;51(Suppl. 1):36-66.
22) Kimer N, Krag A, Moller S, Bendtsen F, Gluud LL. Systematic review with meta-analysis: the effects of rifaximin in hepatic encephalopathy. Aliment Pharmacol Ther 2014;40:123-132.
23) Kalambokis GN, Mouzaki A, Rodi M, Pappas K, Fotopoulos A, Xourgia X, 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) Kalambokis GN, Tsianos EV. Rifaximin reduces endotoxemia and improves liver function and disease severity in patients with decompensated cirrhosis. Hepatology 2012;55:655-656.
25) Vlachogiannakos J, Viazis N, Vasianopoulou P, Vafiadis I, Karamanolis DG, Ladas SD. Long-term administration of rifaximin improves the prognosis of patients with decompensated alcoholic cirrhosis. J Gastroenterol Hepatol 2013;28:450-455.
26) Baik SKL, Cho YL, Kim YZ, Jang MY, Suk Yo, Cheon KT, et al. Rifaximin and propranolol combination therapy is more effective than propranolol monotherapy in the hepatic venous pressure gradient response and propranolol dose reduction—a pilot study. J Hepatol 2015;62(Suppl. 2):S187-S212.
27) Elsass P, Christensen SE, Ranek L, Theilgaard A, Tygstrup N. Continuous reaction time in patients with hepatic encephalopathy. A quantitative measure of changes in consciousness. Scand J Gastroenterol 1981;16:441-447.
28) Weissenborn K, Ennen JC, Schomerus H, Ruckert N, Hecker H. Neuropsychological characterization of hepatic encephalopathy. J Hepatol 2001;34:768-773.
29) Kerlin P, Wong L. Breath hydrogen testing in bacterial overgrowth of the small intestine. Gastroenterology 1988;95:982-988.
30) Moller S, Bendtsen F, Henriksen JH. Effect of volume expansion on systemic hemodynamics and central and arterial blood volume in cirrhosis. Gastroenterology 1995;109:1917-1925.

31) Henriksen JH, Moller S, Fuglsang S, Bendtsen F. Detection of early central circulatory transits in patients with cirrhosis by gamma variate fit of indicator dilution profiles. Am J Physiol Gastrointest Liver Physiol 2005;288:G677-G684.
32) Brinch K, Moller S, Bendtsen F, Becker U, Henriksen JH. Plasma volume expansion by albumin in cirrhosis. Relation to blood volume distribution, arterial compliance and severity of disease. J Hepatol 2003;39:24-31.
33) Brochner-Mortensen J. A simple method for the determination of glomerular filtration rate. Scand J Clin Lab Invest 1972;30: 271-274.
34) Henriksen UL, Henriksen JH. The clearance concept with special reference to determination of glomerular filtration rate in patients with fluid retention. Clin Physiol Funct Imaging 2015; 35:7-16.
35) Krag A, Moller S, Henriksen JH, Holstein-Rathlou NH, Larsen FS, Bendtsen F. Terlipressin improves renal function in patients with cirrhosis and ascites without hepatorenal syndrome. HEPATOLOGY 2007;46:1863-1871.
36) Hobolth L, Moller S, Gronbaek H, Roelsgaard K, Bendtsen F, Feldager Hansen E. Carvedilol or propranolol in portal hypertension? A randomized comparison. Scand J Gastroenterol 2012;47: 467-474.
37) European Medicines Agency. ICH Topic E 6 (R1) guideline for good clinical practice. London, UK; 2002.
38) Bass NM, Mullen KD, Sanyal A, Poordad F, Neff G, Leevy CB, et al. Rifaximin treatment in hepatic encephalopathy. N Engl J Med 2010;362:1071-1081.
39) Bernardi M, Moreau R, Angeli P, Schnabl B, Arroyo V. Mechanisms of decompensation and organ failure in cirrhosis: from peripheral arterial vasodilation to systemic inflammation hypothesis. J Hepatol 2015;63:1272-1284.
40) Vlachogiannakos J, Saveriadis AS, Viazis N, Theodoropoulos I, Foudoulis K, Manolakopoulos S, et al. Intestinal decontamination improves liver haemodynamics in patients with alcohol-related decompensated cirrhosis. Aliment Pharmacol Ther 2009; 29:992-999.
41) Mullen KD, Sanyal AJ, Bass NM, Poordad FF, Sheikh MY, Frederick RT, et al. Rifaximin is safe and well tolerated for long-term maintenance of remission from overt hepatic encephalopathy. Clin Gastroenterol Hepatol 2014;12:1390-1397.
42) Senzolo M, Cholongitas E, Burra P, Leandro G, Thalheimer U, Patch D, et al. Beta-blockers protect against spontaneous bacterial peritonitis in cirrhotic patients: a meta-analysis. Liver Int 2009;29:1189-1193.
43) Sidhu SS, Goyal O, Mishra BP, Sood A, Chhina RS, Soni RK. Rifaximin improves psychometric performance and health-related quality of life in patients with minimal hepatic encephalopathy (the RIME trial). Am J Gastroenterol 2011;106:307-316.

# Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.28898/suppinfo.