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IMPACT OF RIFAXIMIN THERAPY ON ISCHEMIA REPERFUSION INJURY IN LIVER TRANSPLANTATION: A PROPENSITY SCORE-MATCHED ANALYSIS

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Abstract

Intestinal microbiota is thought to play an important role in hepatic ischemia/reperfusion injury (IRI) after liver transplantation (LT). Rifaximin, a nonabsorbable antibiotic used to treat encephalopathy exhibits antibacterial activity within the gut. We report the first study examining the impact of pre-LT rifaximin use on reducing hepatic IRI and inflammatory cell infiltration after LT. This retrospective single-center study included adult LT recipients from January 2013 through June 2016. Patients were divided into 2 groups based on duration of rifaximin use before LT: rifaximin group (≥28 days) and control group (none or <28 days). Patients receiving other antibiotics within 28 days of LT and re-LTs were excluded. Outcomes and messenger RNA (mRNA) expression in the graft were compared by 1:1 propensity score–matching and multivariate analyses. On 1:1 matching (n = 39/group), rifaximin patients had lower postoperative serum transaminase levels and lower early allograft dysfunction (EAD; 10.3% versus 33.3%; P = 0.014). Of the matched patients, 8 patients (n = 4/group) had post-reperfusion liver biopsies (approximately 2h after reperfusion) available for mRNA analysis. Hepatic expression of CD86 (macrophage marker) and cathepsin G (neutrophil marker) was significantly lower in rifaximin patients than controls (P < 0.05). The multivariate analysis included 458 patients. Rifaximin treatment <28 days was identified as an independent risk factor EAD in all patients and those with high Model for End-Stage Liver Disease (MELD) score (MELD ≥35) (n=230). In conclusion, the propensity score–matched and multivariate analyses suggest a therapeutic role of rifaximin in

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The authors have declared that no conflict of interest exists

reducing EAD. Pre-LT rifaximin administration exerted a protective function against early liver injury, potentially by suppressing inflammatory cell activation in the graft.

Keywords

Liver inflammation; Gut microbiota alteration; Early allograft dysfunction; Gut liver axis; Liver injury

Introduction

Liver transplantation (LT) is a well-established treatment for patients with end stage liver disease (1) (2). Hepatic ischemia-reperfusion injury (IRI), resulting from innate immune driven inflammation response, represents the predominant underlying cause of post-transplant organ dysfunction (3) (4) (5) (6). The gut microbiota might be crucial in contributing to hepatic IRI, since the liver has a dual blood supply arising from the hepatic artery and the portal vein, which in turn carries all mesenteric venous blood from gut to the liver.

In humans, gut microbiota are comprised of more than 100 trillion largely colon-restricted, autochthonous bacteria, that not only shape gut morphologic features/mucosal immunity, but also contribute to the development of systemic inflammatory responses (7) (8) (9). A variety of diseases, such as inflammatory bowel disease (10), cardiovascular diseases (11), obesity (12) (13), diabetes (12), colorectal cancer (14), and nervous system diseases (15) are all thought to be affected by gut microbiota. The “gut-liver axis”, widely implicated in the pathogenesis of liver diseases, is increasingly becoming the focus of basic and clinical research (16) (17), including studies on hepatic IRI in rats (18) (19); and hepatic IRI in mouse LT models where a correlation was drawn between gut bacterial density and Kupffer cell density, maturation status and functionality. Hence, suppression of bacterial products might play a therapeutic role in prevention or treatment of hepatic inflammation. One potential therapeutic strategy incorporates antibiotics that can directly act by inhibition of harmful bacterial growth, leading to lower expression of pro-inflammatory cytokines (17) (20).

Rifaximin is a minimally absorbed oral antimicrobial agent that exhibits broad spectrum activity against both aerobic and anaerobic gram-positive and gram-negative microorganisms within the gut, with a low risk of inducing bacterial resistance (21) (22). First approved in the United States in 2004, rifaximin is now used for treatment of hepatic encephalopathy (HE) in many countries (23), improving survival and reducing risk of hospitalization and portal hypertension complications (24) (25). Current American Association for the Study of Liver Diseases guidelines recommend rifaximin as an add-on therapy for the prevention of HE recurrence (26). Among candidates awaiting LT, many patients with history of HE are treated with rifaximin (27) (28). By focusing on the risk of infections in LT recipients, Sun et al (27) showed a protective effect of pre-LT rifaximin use against post-transplant infections with no increase in multidrug-resistant bacterial infections. However, there has been no study linking rifaximin with posttransplant hepatocellular function or early allograft dysfunction (EAD) after LT.

In the present study, we aimed to determine the impact of pre-transplant rifaximin use on post-LT (post reperfusion) liver damage and EAD. In addition, we evaluated inflammatory cell infiltration including neutrophils and macrophages in post-reperfusion liver biopsies since these cells are key players in the development of liver IRI. We hypothesized that pre-LT rifaximin inhibition of harmful bacterial growth within the gut, would result in reduced hepatic IRI and improved outcomes.

Materials and Methods

Patient selection and data collection

This study was approved by the University of California Los Angeles (UCLA) Institutional Review Board. Using a prospectively collected database, we performed a retrospective analysis of adult patients (age ≥ 18 years) who underwent LT from January 2013 through June 2016. 62 patients who underwent re-LT in this period and patients with unavailable data were excluded. Among 458 primary LT patients (n=458), 252 patients underwent pre-LT antibiotics treatment other than rifaximin. 206 patients were ultimately included in the cohort that used in the propensity score-matched analysis. All patients received prophylactic antibiotic therapy and immunosuppressive therapy per our institutional protocol during the perioperative period. All liver grafts, procured from donation after brain death or cardiac death with standardized techniques were stored in cold University of Wisconsin solution prior to implantation. We collected data including rifaximin and other preoperatively administrated antibiotics, recipient pretransplant demographics (age, gender, race, past history, indication for LT, length of pre-transplant hospital stay, and model for end-stage liver disease (MELD) score), donor characteristics (age, gender, race, cause of death, donation after circulatory death (DCD), past history, donor risk index (DRI), and laboratory data), cold ischemia time (CIT), warm ischemia time (WIT), and patient outcomes, including postoperative laboratory data in the first 7 post-LT days. Serum transaminase levels were used as a surrogate marker of hepatocellular injury. EAD was defined by the presence of one or more of the following; bilirubin level of ≥ 10 mg/dl on POD 7, prothrombin time-international normalized ratio (PT-INR) ≥ 1.6 on POD 7, or aspartate transaminase (AST) and alanine transaminase (ALT) levels of > 2000 IU/L within the first 7 days.

Patients were grouped into the rifaximin or control arm based on the presence or absence of daily rifaximin administration for the optimal duration in days. To identify the optimal duration of rifaximin in days needed to exert a beneficial effect, sensitivity and specificity for prevention of EAD were analyzed by receiver operating characteristic (ROC) curve at multiple time points; 1 day, 5 days, 7 days (1 week), 10 days, 14 days (2 weeks), 21 days (3 weeks), 28 days (4 weeks), 42 days (6 weeks), and 56 days (8 weeks) (table S1). Rifaximin was administrated at a dose of 550 mg twice daily.

Human liver sample collection and quantitative polymerase chain reaction (PCR) analysis

Protocol Tru-Cut (CareFusion, SanDiego, CA) needle biopsies (Bx) were obtained intra-operatively from the left lobe approximately 2 h after portal reperfusion (prior to surgical closure of abdomen) and snap-frozen as previously described (29) (30). RNA extracted with

RNAse Mini Kit (Qiagen, Germantown, MD) was reverse-transcribed into cDNA. Quantitative PCR was performed using Quant Studio 3 (Applied Biosystems, Foster City, CA). The primer sequences are listed in table S2. The expression of the target gene was normalized to the housekeeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Statistical analysis

Descriptive statistics were reported for the entire study cohort. Categorical variables were summarized as numbers and percentages, and continuous variables were summarized as medians and ranges. Groups were compared using Pearson's Chi-square / Fisher test for categorical variables and the Mann-Whitney U test for continuous variables. The propensity matching method (1:1) was used to control confounding factors and selection bias between the rifaximin and control groups. Survival curves were generated by the Kaplan–Meier method, and differences in survival rates were analyzed using the Log rank test. To identify risk factors of EAD, logistic regression modelling was used and all significant variables in univariate analyses were subsequently included in the multivariate analysis. All tests were two-sided, and $p < 0.05$ was considered statistically significant. All analyses were performed using IBM® SPSS® Statistics version 25 (IBM Corporation, Armonk, NY).

Results

Demographics of the patient cohort (n=206) after exclusion of patients with other antibiotics treatment than rifaximin and re-LT patients are shown in table 1. The median recipient age was 60 (20–75), with males constituting approximately 70 % of the patients. The most common recipient race was Hispanic (n = 95, 46.1 %), followed by Whites (n = 68, 33.0 %). Hepatitis C virus (HCV) related hepatic disease was the most predominant indication for LT (n = 107, 51.9 %), followed by nonalcoholic steatohepatitis (NASH) (n = 28, 13.6 %) and alcohol (ethanol, EtOH) related liver disease (n = 23, 11.2 %). Concomitant hepatocellular carcinoma (HCC) occurred in 128 patients (62.1%). The median (range) MELD score was 14 (6–47). Donor median age (range) was 38 (7–76) and approximately 60 % of the donors were male. Almost 50 % of donors were Whites (n = 102, 49.5%) and the rate of DCD donor graft use was 4.4 % (n = 9). Median CIT was 440 minutes and median warm ischemia time (WIT) was 48 minutes.

Classification according to rifaximin therapy

We selected 28 days as cut-off value since it had the maximum sum of sensitivity and specificity within its candidates. Forty-nine patients (rifaximin group, 23.8%) received rifaximin for 28 days or more prior to LT, and the remaining 157 patients were rifaximin-free or received rifaximin for 1–27 days (control group). The perioperative clinical features of 206 patients were analyzed according to the presence or absence of continuous administration of rifaximin for at least 28 days before LT (table 2).

As shown in table 2, recipient race distribution, indication for LT, presence of concomitant HCC, MELD score, and distribution of donor race were significantly different between two groups before matching ($p < 0.05$). The rate of patients who had HCC in the rifaximin group was significantly lower than those in control group (38.7% versus 69.4%, $p < 0.001$).

Patients in the rifaximin group had significantly higher MELD scores than those in the control group [28 (9–43) versus 13 (6–47), $p < 0.001$]. There was no significant difference in pre-transplant serum transaminase levels (AST: 59 (20–197) versus 59 (20–1918) IU/l, $p = 0.963$, ALT: 29 (11–134) versus 33 (9–3705), $p = 0.125$). Post-transplant transaminase levels and the rate of EAD before matching are shown in supplemental data (figure S1, S2). Serum ALT levels at / from POD 0 (approximately 6 h after operation) to POD7 and AST levels at POD 0–4, 6–7 in the rifaximin group were significantly lower than those in control group ($p < 0.05$). The rate of EAD was also significantly lower in the rifaximin group (14.3 % versus 29.9 %, $p = 0.030$).

Propensity matching analysis between rifaximin and control group

The rifaximin and control groups were matched in a 1:1 ratio to the nearest propensity scores and 39 patients in each group were selected. Table 3 shows the results comparing the two groups after propensity matching. This controlled for all significant differences in recipient and donor factors between the two groups. After the propensity matching, the value of post-transplant serum transaminase levels and the rate of EAD were compared between the rifaximin and control groups. As shown in figure 1, patients in the rifaximin group had significantly lower serum AST and ALT levels at POD 0. Moreover, serum ALT levels at POD2-POD6 in the rifaximin group were significantly lower than those in control group ($p < 0.05$). Despite similar trends, serum levels of AST at POD1–7 and ALT at POD1, 7 did not reach statistical significance. Notably, the rifaximin group had a significantly lower rate of EAD after matching compared to the control group as was the case before matching (10.3 % versus 33.3 %, $p = 0.014$, figure 2). There were no differences in patient and graft survival between the two groups (figure S3).

Evaluation of inflammatory cell activation in post-reperfusion LT

Liver Bx samples, obtained approximately 2h after portal reperfusion from four patients in each group after matching (figure 3), were evaluated for the activation of infiltrating inflammatory neutrophils and macrophages, as well as screening mRNA coding for CD86, CD68 and Cathepsin G. CD86 is a co-stimulatory molecule on monocytic cells, including macrophages; CD68 is a macrophage marker; while Cathepsin G is typically stored in neutrophils and is released by a variety of stimuli, including IR-stress. As shown in figure 4, the expression of mRNA coding for CD86 and Cathepsin G in the rifaximin group was significantly lower than in the control group (CD86: 0.16 ± 0.02 versus 0.26 ± 0.02, $p=0.029$, Cathepsin G: 0.09 ± 0.01 versus 0.16 ± 0.02, $p=0.029$) while CD68 expression despite showing similar trends (0.12 ± 0.02 versus 0.19 ± 0.03, $p = 0.200$) did not reach statistical significance between the two groups.

Uni- and multivariate analysis for early allograft dysfunction in all patients and patients with high MELD scores

Univariate and multivariate analysis were used to determine the impact of pre-LT rifaximin treatment on EAD in all patients (n=458), as well as high MELD patients (MELD≥35, n=230) only. On univariate analysis for all patients, longer CIT/ WIT, older donor age, higher donor BMI, higher DRI, and rifaximin treatment < 28 days were identified as risk factors for the development of EAD ($p<0.05$, table S3). On Univariate analysis in high

MELD patients longer WIT, older donor age, higher donor BMI, and rifaximin treatment < 28 days were identified as risk factors for the development of EAD ($p<0.05$, table S4).

On multivariate analysis for all patients, rifaximin treatment < 28 days (Odds ratio [OR]: 2.096, 95% Confidence interval [CI]: 1.298–3.382, p-0.002) and longer CIT (OR: 1.003, 95% CI: 1.002–1.005, $p<0.001$) were identified independent risk factors for EAD (table 4). Additionally, multivariate analysis in patients with high MELD scores showed that rifaximin treatment < 28 days (OR: 2.015, 95% CI: 1.071–3.792, $p=0.030$) and longer WIT (OR: 1.052, 95% CI: 1.026–1.079, $p<0.001$) were independent risk factors for EAD (Table 5).

Discussion

IRI is associated with acute cellular damage, cell death, and a severe hepatocellular inflammatory response (31) (32) (33). EAD considered to be largely due to IRI, is associated with increased graft failure and mortality. In this retrospective propensity score-matched analysis, we document that pre-operative rifaximin treatment improved hepatocellular function after LT. Patients who received continuous rifaximin therapy for at least 28 days prior to surgery had diminished serum AST/ALT levels and lower rates of EAD post-LT. To the best of our knowledge, this study is the first to report the influence of rifaximin therapy on post-transplant graft function in LT recipients. Since the elevation of serum transaminases is often used as a surrogate marker of hepatic IRI (34) (35), our results indicate that unlike controls, rifaximin attenuated IRI in LT.

In the propensity score matched analysis (n=206), there were some notable differences in patient demographics; patients in the rifaximin group had higher MELD scores and a lower rate of concomitant HCC, compared to control. Consistent with a previous report examining the protective effect of rifaximin against post-transplant infections (27), our study showed that patients with pre-transplant rifaximin treatment had a higher acuity level reflected by significantly higher MELD scores compared to controls. Another study (36) by Wong et al. reported that patients with a history of HE had higher MELD scores compared to patients without HE. In the present study, the rate of patients with a history of HE in the rifaximin group was significantly higher than that in the control group (13.4% vs 100%, $p<0.001$). These previous studies support the observed MELD Score differences in our cohort before matching. Additionally, there was a lower incidence of HCC among patients treated with rifaximin. This is consistent with previous reports (27) (28), and is not surprising since most patients awaiting LT for HCC have relatively compensated liver function and thus do not suffer from HE. This also accounts for the difference in MELD score between the two groups. These studies as well as the present analysis reflect the inherent medical acuity differences between patients likely to be on rifaximin and those who do not medically require it prior to LT. This important difference however was mitigated by use of propensity score matching analysis in order to verify the true effect of rifaximin.

We observed no differences between donor graft quality in any of the donor parameters measured including DRI before or after propensity matching. This is noteworthy given the possible confounding effect resulting from potentially using better quality grafts for the higher acuity recipients and grafts more likely to be predisposed to IRI in the lower acuity

patients with HCC or those well enough not to require rifaximin pre-transplant. Furthermore, our matching model reduced differences including donor factors between the two groups.

IRI may occur at several key time points of LT, starting in the donor, continuing during cold storage, and when the organ is reperfused with the recipient's blood. During these events, not only do donor-derived damage-associated molecular patterns (DAMPS), which are mainly released by damaged liver cells readily stimulate the immune system, but so do pathogen-associated molecular patterns (PAMPS), primarily secreted from recipient gut microbiota. These events all contribute to the severity of IRI (33). The effect of pre-transplant rifaximin administration is considered to impact liver IRI at the time of hepatic reperfusion.

In attempting to elucidate the putative mechanism of rifaximin-mediated hepatoprotection against IRI, we focused on inflammatory cell graft infiltrates, since neutrophils and macrophages are key players in the pathophysiology of liver IRI (4) (5) (6). The mRNA levels coding for CD86 and Cathepsin G, a marker of macrophages and neutrophils respectively, were lower in LT of rifaximin treated vs. control patients. Patients with end-stage liver disease have a relatively low concentration of bile acids in the gut; this is believed to contribute to chronic inflammation due to an overgrowth of pathogenic bacteria, increased endotoxin levels, and secondary stimulation of a potent inflammatory response (37) (38). Numerous studies indicate that inflammatory cells such as neutrophils and macrophages respond to bacterial products via NF-κB and production of pro-inflammatory cytokines / chemokines, suggesting that these cells would be responsive to physiologically relevant levels of microbial products that reach the liver (39). Rifaximin directly affects bacterial growth, leading to a lower pro-inflammatory response (16) (17). In a recent study examining patients with Non Alcoholic Fatty Liver Disease (NAFLD), 28 days of treatment with rifaximin was shown to exert beneficial effects in early clinical trials, lowering endotoxemia and reducing transaminases (40). Another study in HE patients suggested that rifaximin therapy has a systemic and local effect on the microbiota, metabolome, endotoxemia and cognition, and a significant improvement in endotoxemia was observed with a modest change in stool microbiota composition (37). Hence, the mechanism of action of rifaximin on hepatic IRI, based on these studies and our current findings, may involve modulation of microbiota inflammatory function leading to reducing neutrophil / macrophage activation in IR-stressed liver grafts.

Furthermore, it is suspected that an innate immune pathologic response occurs with subsequent bacterial translocation of organisms from the gut in patients with a history of HE and portal hypertension that may result in chronic endotoxemia (41). This cascade culminates in a local milieu of pro-inflammatory cytokines/chemokines which upregulate adhesion receptors and activate neutrophils (41). Although there is no reliable evidence regarding the difference in the inflammatory condition of HE patients before and during administration of rifaximin compared to patients without HE, patients in the rifaximin group might undergo LT with a high degree of underlying inflammation. Nevertheless, lower levels of neutrophil / macrophage infiltration in their IR-stressed livers may support the protective effect of rifaximin against gut-derived hepatic inflammation.

Some limitations to this study include the fact that it is a single center retrospective analysis, thereby providing for an inherent difference in the presence of HE between the two groups even after matching. The median (range) MELD score of all 78 recipients included in matching study was 21 (6–43), i.e., relatively low compared with a median MELD of 35 (6–51) for 458 primary LT patients at our institution during the study period. This is mainly a result of excluding many high MELD patients requiring concomitant antibiotic therapy other than rifaximin for infections, such as spontaneous bacterial peritonitis or septic shock. Unfortunately propensity score-matching in all patients was not possible since the differences between the groups were too large to properly match (table S5). Therefore, we used multivariate analysis for EAD to determine the impact of rifaximin treatment. Multivariate analysis identified rifaximin use < 28 days as an independent risk factor for EAD. This finding was also present in the high MELD patient cohort suggesting that rifaximin treatment may potentially protect against liver injury in high MELD patients as well. In fact, while we used 28 days of rifaximin treatment as the minimum duration of therapy based on ROC curve and previous reports (24) (40), the rate of patients receiving rifaximin for at least 1 day was nearly 60 % at our institution (data not shown). Therefore, although rifaximin use may have provided a protective benefit against IRI and EAD in those patients, this was difficult to ascertain given the simultaneous use of other antibacterial agents in those patients.

The exact effect of rifaximin administration on graft injury and its optimal duration in high acuity patients are yet to be fully delineated. To better evaluate the effect of sole pre-transplant rifaximin administration, prospective studies, including randomized controlled trials, are needed. It is also important to note that in the present study, rifaximin was used only in the pre-transplant period and was not continued post-transplant. Whether continuing administration of rifaximin in the immediate postoperative period can further decrease the rate of EAD after LT or not is unknown. Further experiments in animal LT models might assist in developing a better mechanistic appreciation of the specific molecular signaling pathways by which rifaximin may exert this protective function.

In conclusion, pretransplant rifaximin administration exerted a protective effect against EAD, while suppressing neutrophil / macrophage activation in IR-stressed human LT. These propensity score-matched and multivariate analyses suggest a therapeutic potential for pre-operative gut microbiota alteration by rifaximin against IRI in LT patients. Additional studies are needed to further elucidate this relationship and analyze underlying mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviation

ALF	acute liver failure
ALT	alanine transaminase
AST	aspartate transaminase
Bx	biopsy
CIT	cold ischemia time
DAMPS	donor-derived damage-associated molecular patterns
DCD	donation after circulatory death
DRI	donor risk index
EAD	early allograft dysfunction
EtOH	ethanol
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HE	hepatic encephalopathy
IRI	ischemia reperfusion injury
LT	liver transplantation
MELD	model for end-stage liver disease
NAFLD	nonalcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
PAMPS	pathogen-associated molecular patterns
PCR	polymerase chain reaction
POD	postoperative day
PT-INR	prothrombin time international normalized ratio
ROC	receiver operating characteristic
UCLA	University of California Los Angeles
WIT	warm ischemia time

References

1. Starzl TE, Marchioro TL, Vonkaulla KN, Hermann G, Brittain RS, Waddell WR. HOMOTRANSPLANTATION OF THE LIVER IN HUMANS. Surg Gynecol Obstet 1963;117:659–676. [PubMed: 14100514]
2. Agopian VG, Petrowsky H, Kaldas FM, Zarrinpar A, Farmer DG, Yersiz H, Holt C, et al. The evolution of liver transplantation during 3 decades: analysis of 5347 consecutive liver transplants at a single center. Ann Surg 2013;258:409–421. [PubMed: 24022434]
3. Serracino-Inglott F, Habib NA, Mathie RT. Hepatic ischemia-reperfusion injury. Am J Surg 2001;181:160–166. [PubMed: 11425059]
4. Uchida Y, Ke B, Freitas MC, Yagita H, Akiba H, Busuttil RW, Najafian N, et al. T-cell immunoglobulin mucin-3 determines severity of liver ischemia/reperfusion injury in mice in a TLR4-dependent manner. Gastroenterology 2010;139:2195–2206. [PubMed: 20637206]
5. Zhai Y, Busuttil RW, Kupiec-Weglinski JW. Liver ischemia and reperfusion injury: new insights into mechanisms of innate-adaptive immune-mediated tissue inflammation. Am J Transplant 2011;11:1563–1569. [PubMed: 21668640]
6. Zhai Y, Petrowsky H, Hong JC, Busuttil RW, Kupiec-Weglinski JW. Ischaemia-reperfusion injury in liver transplantation--from bench to bedside. Nat Rev Gastroenterol Hepatol 2013;10:79–89. [PubMed: 23229329]
7. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 2006;124:837–848. [PubMed: 16497592]
8. Bouskra D, Brezillon C, Berard M, Werts C, Varona R, Boneca IG, Eberl G. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. Nature 2008;456:507–510. [PubMed: 18987631]
9. Smith K, McCoy KD, Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin Immunol 2007;19:59–69. [PubMed: 17118672]
10. Seksik P, Sokol H, Lepage P, Vasquez N, Manichanh C, Mangin I, Pochart P, et al. Review article: the role of bacteria in onset and perpetuation of inflammatory bowel disease. Aliment Pharmacol Ther 2006;24 Suppl 3:11–18. [PubMed: 16961738]
11. Tang WH, Hazen SL. The contributory role of gut microbiota in cardiovascular disease. J Clin Invest 2014;124:4204–4211. [PubMed: 25271725]
12. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008;57:1470–1481. [PubMed: 18305141]
13. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58:1091–1103. [PubMed: 19240062]
14. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol 2014;12:661–672. [PubMed: 25198138]
15. Petra AI, Panagiotidou S, Hatziagelaki E, Stewart JM, Conti P, Theoharides TC. Gut-Microbiota-Brain Axis and Its Effect on Neuropsychiatric Disorders With Suspected Immune Dysregulation. Clin Ther 2015;37:984–995. [PubMed: 26046241]
16. Wiest R, Albillos A, Trauner M, Bajaj JS, Jalan R. Targeting the gut-liver axis in liver disease. J Hepatol 2017;67:1084–1103. [PubMed: 28526488]
17. Chassaing B, Etienne-Mesmin L, Gewirtz AT. Microbiota-liver axis in hepatic disease. Hepatology 2014;59:328–339. [PubMed: 23703735]
18. Xing HC, Li LJ, Xu KJ, Shen T, Chen YB, Sheng JF, Yu YS, et al. Intestinal microflora in rats with ischemia/reperfusion liver injury. J Zhejiang Univ Sci B 2005;6:14–21. [PubMed: 15593386]
19. Corbitt N, Kimura S, Isse K, Specht S, Chedwick L, Rosborough BR, Lunz JG, et al. Gut bacteria drive Kupffer cell expansion via MAMP-mediated ICAM-1 induction on sinusoidal endothelium and influence preservation-reperfusion injury after orthotopic liver transplantation. Am J Pathol 2013;182:180–191. [PubMed: 23159949]

20. Fujisaka S, Ussar S, Clish C, Devkota S, Dreyfuss JM, Sakaguchi M, Soto M, et al. Antibiotic effects on gut microbiota and metabolism are host dependent. J Clin Invest 2016;126:4430–4443. [PubMed: 27775551]
21. Gillis JC, Brogden RN. Rifaximin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic potential in conditions mediated by gastrointestinal bacteria. Drugs 1995;49:467–484. [PubMed: 7774516]
22. Debbia EA, Maioli E, Roveta S, Marchese A. Effects of rifaximin on bacterial virulence mechanisms at supra- and sub-inhibitory concentrations. J Chemother 2008;20:186–194. [PubMed: 18467244]
23. Scarpignato C, Pelosini I. Experimental and clinical pharmacology of rifaximin, a gastrointestinal selective antibiotic. Digestion 2006;73 Suppl 1:13–27. [PubMed: 16498249]
24. 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. [PubMed: 23216382]
25. Bass NM, Mullen KD, Sanyal A, Poordad F, Neff G, Leevy CB, Sigal S, et al. Rifaximin treatment in hepatic encephalopathy. N Engl J Med 2010;362:1071–1081. [PubMed: 20335583]
26. Vilstrup H, Amodio P, Bajaj J, Cordoba J, Ferenci P, Mullen KD, Weissenborn K, et al. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. Hepatology 2014;60:715–735. [PubMed: 25042402]
27. Sun HY, Wagener M, Cacciarelli TV, Singh N. Impact of rifaximin use for hepatic encephalopathy on the risk of early post-transplant infections in liver transplant recipients. Clin Transplant 2012;26:849–852. [PubMed: 22432742]
28. Esfeh JM, Hanouneh IA, Koval CE, Kovacs C, Dalal DS, Ansari-Gilani K, Confer BD, et al. Impact of pretransplant rifaximin therapy on early post-liver transplant infections. Liver Transpl 2014;20:544–551. [PubMed: 24493238]
29. Kageyama S, Nakamura K, Fujii T, Ke B, Sosa RA, Reed EF, Datta N, et al. Recombinant relaxin protects liver transplants from ischemia damage by hepatocyte glucocorticoid receptor: From bench-to-bedside. Hepatology 2018;68:258–273. [PubMed: 29350771]
30. Nakamura K, Kageyama S, Yue S, Huang J, Fujii T, Ke B, Sosa RA, et al. Heme oxygenase-1 regulates sirtuin-1-autophagy pathway in liver transplantation: From mouse to human. Am J Transplant 2018;18:1110–1121. [PubMed: 29136322]
31. Friedman BH, Wolf JH, Wang L, Putt ME, Shaked A, Christie JD, Hancock WW, et al. Serum cytokine profiles associated with early allograft dysfunction in patients undergoing liver transplantation. Liver Transpl 2012;18:166–176. [PubMed: 22006860]
32. Busuttil RW, Tanaka K. The utility of marginal donors in liver transplantation. Liver Transpl 2003;9:651–663. [PubMed: 12827549]
33. Sosa RA, Zarrinpar A, Rossetti M, Lassman CR, Naini BV, Datta N, Rao P, et al. Early cytokine signatures of ischemia/reperfusion injury in human orthotopic liver transplantation. JCI Insight 2016;1:e89679.
34. Robertson FP, Bessell PR, Diaz-Nieto R, Thomas N, Rolando N, Fuller B, Davidson BR. High serum Aspartate transaminase levels on day 3 postliver transplantation correlates with graft and patient survival and would be a valid surrogate for outcome in liver transplantation clinical trials. Transpl Int 2016;29:323–330. [PubMed: 26615011]
35. Leithead JA, Armstrong MJ, Corbett C, Andrew M, Kothari C, Gunson BK, Muiesan P, et al. Hepatic ischemia reperfusion injury is associated with acute kidney injury following donation after brain death liver transplantation. Transpl Int 2013;26:1116–1125. [PubMed: 24033747]
36. Wong RJ, Aguilar M, Gish RG, Cheung R, Ahmed A. The impact of pretransplant hepatic encephalopathy on survival following liver transplantation. Liver Transpl 2015;21:873–880. [PubMed: 25902933]
37. Ridlon JM, Alves JM, Hylemon PB, Bajaj JS. Cirrhosis, bile acids and gut microbiota: unraveling a complex relationship. Gut Microbes 2013;4:382–387. [PubMed: 23851335]

38. Bajaj JS, Heuman DM, Sanyal AJ, Hylemon PB, Sterling RK, Stravitz RT, Fuchs M, et al. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. PLoS One 2013;8:e60042.

39. Su GL, Klein RD, Aminlari A, Zhang HY, Steinstraesser L, Alarcon WH, D.G. R, et al. Kupffer cell activation by lipopolysaccharide in rats: role for lipopolysaccharide binding protein and toll-like receptor 4. Hepatology. 2000;31:932–936. [PubMed: 10733550]

40. Gangarapu V, Ince AT, Baysal B, Kayar Y, Kilic U, Gok O, Uysal O, et al. Efficacy of rifaximin on circulating endotoxins and cytokines in patients with nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2015;27:840–845. [PubMed: 26043290]

41. Bode C, Kugler V, Bode JC. Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with no evidence of chronic liver disease following acute alcohol excess. J Hepatol 1987;4:8–14. [PubMed: 3571935]

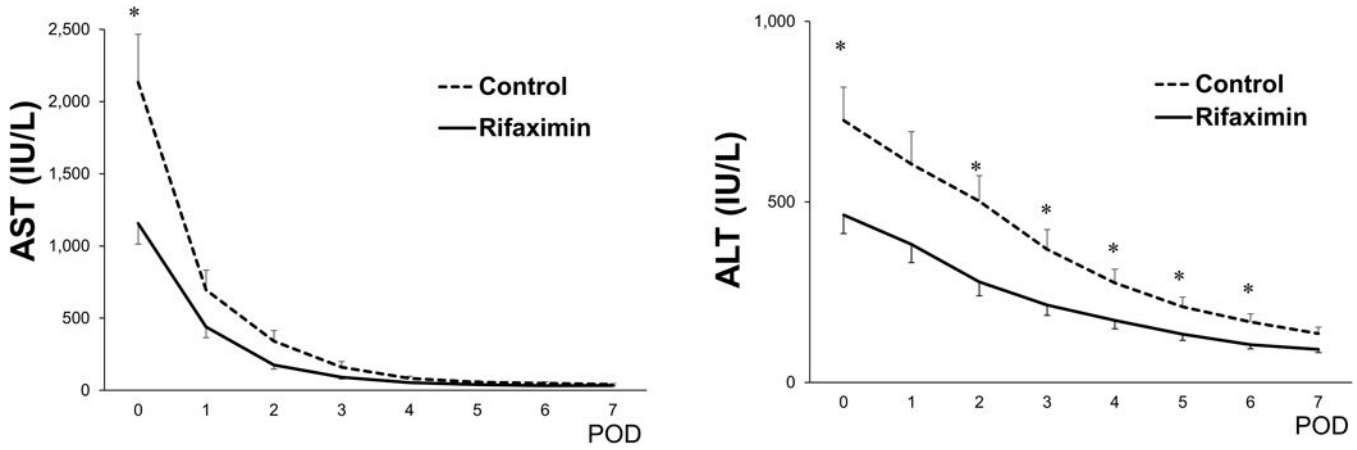

Figure 1. Comparison of post-transplant transaminase levels after propensity matching.

Serum AST levels at POD 0 and serum ALT levels at POD 0 and POD 2-6 in the rifaximin group were significantly lower than those in controls. * $p < 0.05$

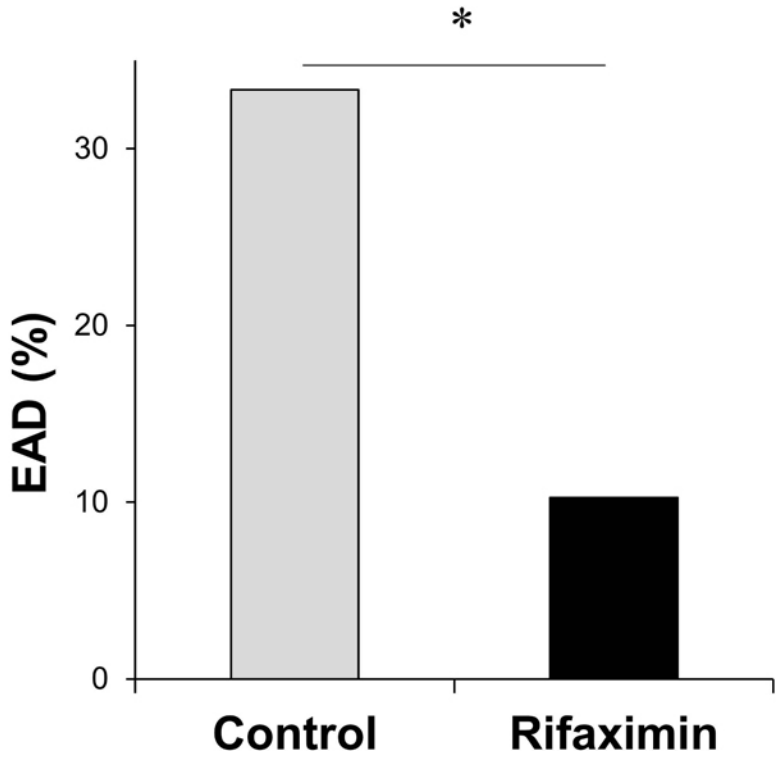

Figure 2. The rate of early allograft dysfunction in patients after propensity matching.
The rate of EAD in rifaximin group was significantly lower than the control group. * $p < 0.05$

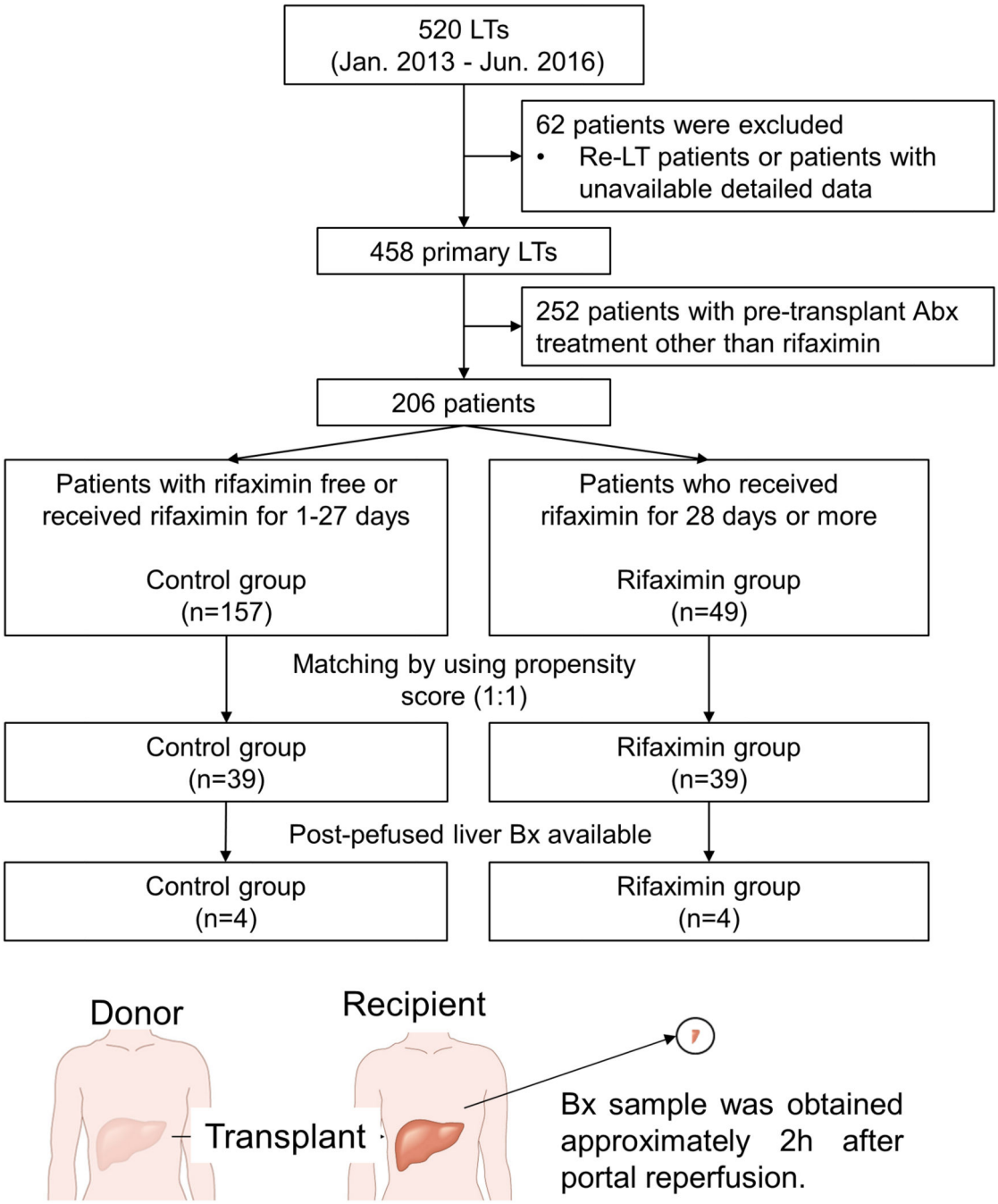

Figure 3.
Flow chart showing study design and the population of patients included in the analysis.

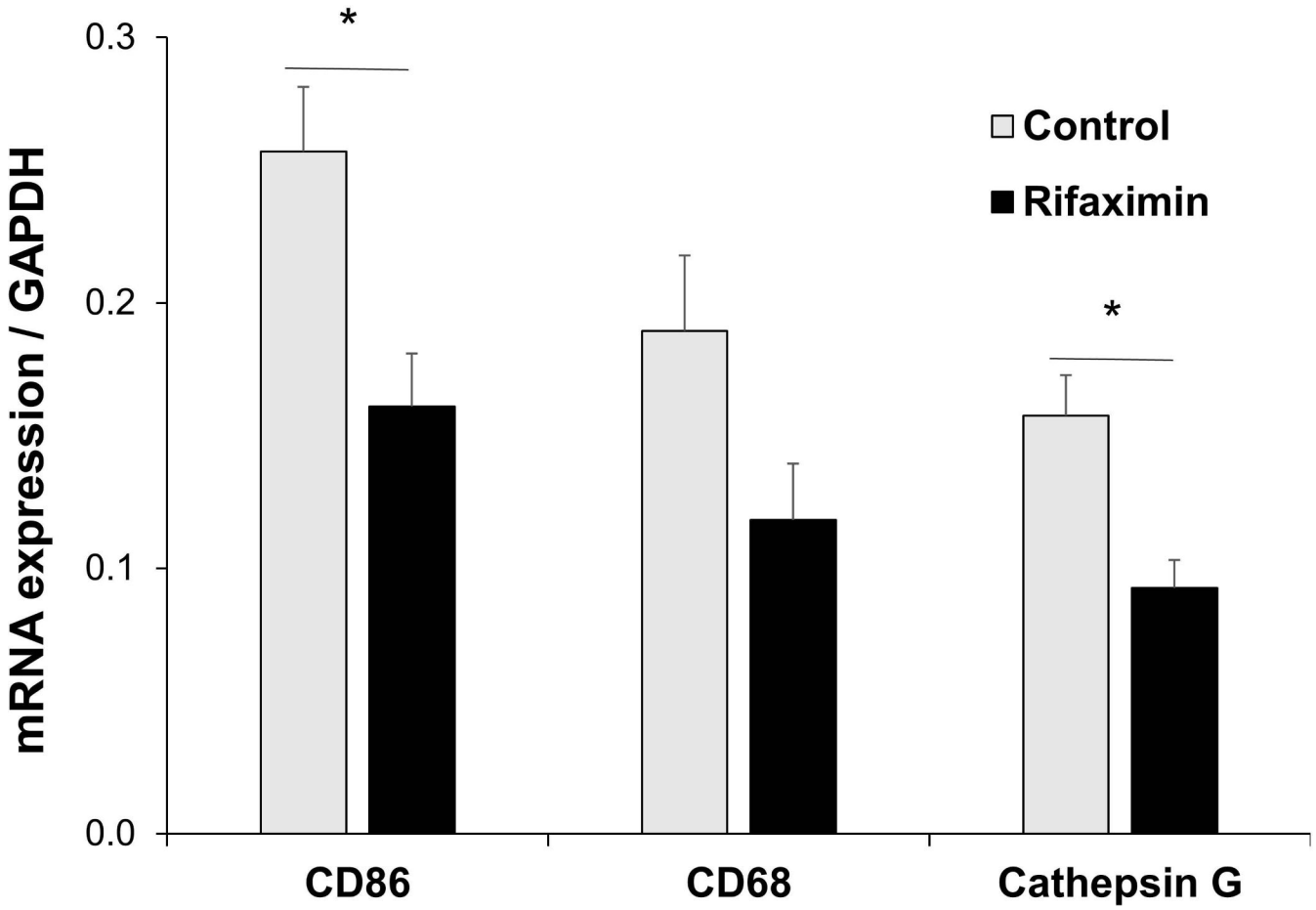

Figure 4. mRNA expression of neutrophil and macrophage markers.

The rifaximin group showed significantly lower expression of hepatic CD86 and Cathepsin G compared to controls. CD68 expression levels in the rifaximin group showed tendency to be lower than those measured in the control group. n = 4/ group, *p < 0.05

Table1.

Patient demographics

Variables	Whole patients (n=206)
Recipient age (y. o.)	60 (20–75)
Recipient gender (female / male)	59 (28.6%) / 147 (71.4%)
Recipient race	
White	68 (33.0%)
Hispanic	95 (46.1%)
American African	14 (6.8%)
Asian	22 (10.7%)
Others	7 (3.4%)
Indication of LT	
HBV	13 (6.3%)
HCV	107 (51.9%)
EtOH	23 (11.2%)
NASH	28 (13.6%)
ALF	9 (4.4%)
Others	26 (12.6%)
Concomitant HCC	128 (62.1%)
MELD	14 (6–47)
Donor Age (y. o.)	38 (7–76)
Donor gender (female / male)	78 (37.9%) / 128 (62.1 %)
Donor race	
White	102 (49.5 %)
Hispanic	64 (31.1%)
American African	25 (12.1%)
Asian	12 (5.8%)
Others	3 (1.5%)
DCD	9 (4.4%)
Donor risk index	1.4 (0.9–2.8)
Cold ischemia time (min)	440 (163–878)
Warm ischemia time (min)	48 (23–176)

Table 2.
Comparison between rifaximin and control groups before matching.

Forty-nine patients (rifaximin group) received rifaximin for 28 days or more prior to LT, and the other 157 patients (control group) did not. Race of recipient and donor, were significant different between two groups ($p < 0.05$). In the rifaximin group, the rate of HCC was lower and MELD score was significantly higher, as compared to control group ($p < 0.001$).

Variables	Control (n=157)	Rifaximin (n=49)	P value
Recipient age (y. o)	60 (20–75)	59 (21–68)	0.095
Recipient gender (female / male)	47 (29.9%) / 110 (70.1%)	12 (24.5%) / 37 (75.5%)	0.462
Recipient race			**0.044**
White	58 (36.9%)	10 (20.4%)	
Hispanic	67 (42.7%)	28 (57.1%)	
American African	12 (7.6%)	2 (4.1%)	
Asian	17 (10.8%)	5 (10.2%)	
Others	3 (1.9%)	4 (8.2%)	
Indication of OLT			**0.016**
HBV	13 (8.3%)	0	
HCV	83 (52.9%)	24 (49.0%)	
EtOH	14 (8.9%)	9 (18.4%)	
NASH	17 (10.8%)	11 (22.4%)	
ALF	9 (5.7%)	0	
Others	21 (13.4%)	5 (10.2%)	
Past history of recipient			
Smoking	73 (46.5%)	22 (44.9%)	0.845
Hypertension	73 (46.5%)	23 (46.9%)	0.957
Diabetes	55 (35.0%)	17 (34.7%)	0.965
Coronary artery disease	24 (15.3%)	11 (22.4%)	0.244
Concomitant HCC	109 (69.4)	19 (38.7)	**<0.001**
Pretransplant AST (IU/L)	59 (20–1918)	59 (20–197)	0.963
Pretransplant ALT (IU/L)	33 (9–3705)	29 (11–134)	0.125
MELD	13 (6–47)	28 (9–43)	**<0.001**
Preoperative hospital stay (days)	1 (0–136)	1 (0–48)	0.159
Preoperative ICU stay	16 (10.2%)	6 (12.2%)	0.684
Donor age (y. o.)	38 (7–76)	38 (12–70)	0.973
Donor gender (female / male)	57 (36.3%) / 100 (63.7%)	21 (42.9%) / 28 (57.1%)	0.409
Donor race			**0.002**
White	77 (49.0%)	25 (51.0%)	
Hispanic	54 (34.4%)	10 (20.4%)	
American African	13 (8.3%)	12 (24.5%)	
Asian	12 (7.6%)	0	
Others	1 (0.6%)	2 (4.1%)	
Donor cause of dead			0.435

Variables	Control (n=157)	Rifaximin (n=49)	P value
Head trauma	60 (38.2%)	14 (28.6%)	
Cerebrovascular accident	59 (37.6%)	20 (40.8%)	
Anoxia	38 (24.2%)	15 (30.6%)	
DCD	6 (3.8%)	3 (6.1%)	0.492
Donor past history			
Hypertension	47 (29.9%)	17 (34.7%)	0.530
Diabetes	18 (11.5%)	9 (18.4%)	0.211
Coronary artery disease	7 (4.5%)	3 (6.1%)	0.636
Donor AST (IU/L)	43 (9–747)	38 (13–294)	0.390
Donor ALT (IU/L)	35 (7–286)	31 (9–165)	0.273
Donor T-Bil (mg/dl)	0.8 (0.2–11.5)	0.7 (0.2–2.7)	0.864
Donor PT-INR	1.2 (0.9–16.1)	1.2 (0.9–2.8)	0.933
Donor Risk Index	1.5 (0.9–2.3)	1.4 (1.0–2.8)	0.565
Cold ischemia time (min)	439 (163–878)	463 (211–760)	0.970
Warm ischemia time (min)	48 (23–176)	49 (27–81)	0.185

Table 3.
Comparison between rifaximin and control groups after propensity matching.

The rifaximin and control groups were matched in a 1:1 and 39 patients in both groups were selected. The propensity matching reduced differences between the 2 groups that existed before the matching.

Variables	Control (n=39)	Rifaximin (n=39)	P value
Recipient age (y. o)	57 (21–72)	59 (37–68)	0.519
Recipient gender (female / male)	9 (23.1%) / 30 (76.9%)	11 (28.2%) / 28 (71.8%)	0.604
Recipient race			0.986
White	10 (25.6%)	10 (25.6%)	
Hispanic	21 (53.8%)	20 (51.3%)	
American African	1 (2.6%)	2 (5.1%)	
Asian	4 (10.3%)	4 (10.3%)	
Others	3 (7.7%)	3 (7.7%)	
Indication of OLT			0.106
HBV	4 (10.3%)	0	
HCV	23 (59.0%)	20 (51.3%)	
EtOH	4 (10.3%)	5 (12.8%)	
NASH	3 (7.7%)	10 (25.6%)	
ALF	4 (10.3%)	4 (10.3%)	
Others	1 (2.6%)	0	
Past history of recipient			
Smoking	23 (59.0%)	18 (46.2%)	0.257
Hypertension	16 (41.0%)	16 (41.0%)	>0.999
Diabetes	14 (35.9%)	12 (30.8%)	0.631
Coronary artery disease	4 (10.3%)	8 (20.5%)	0.209
Concomitant HCC	22 (56.4%)	18 (46.2%)	0.365
Pretransplant AST (IU/L)	50 (20–885)	63 (20–197)	0.285
Pretransplant ALT (IU/L)	30 (9–1170)	31 (11–134)	0.780
MELD	17 (6–43)	25 (9–40)	0.206
Preoperative hospital stay (days)	1 (0–35)	1 (0–47)	0.726
Preoperative ICU stay	5 (12.8%)	4 (10.3%)	0.723
Donor age (y. o.)	36 (8–65)	35 (12–70)	0.853
Donor gender (female / male)	13 (33.3%) / 26 (66.7%)	17 (43.6%) / 22 (56.4%)	0.352
Donor race			0.541
White	17 (43.6%)	21 (53.8%)	
Hispanic	14 (35.9%)	10 (25.6%)	
American African	7 (17.9%)	8 (20.5%)	
Asian	0	0	
Others	1 (2.6%)	0	
Donor cause of dead			0.961
Head trauma	14 (35.9%)	13 (33.3%)	
Cerebrovascular accident	14 (35.9%)	14 (35.9%)	

Variables	Control (n=39)	Rifaximin (n=39)	P value
Anoxia	11 (28.2%)	12 (30.8%)	
DCD	3 (7.7%)	2 (5.1%)	0.644
Donor past history			
Hypertension	13 (33.3%)	13 (33.3%)	>0.999
Diabetes	3 (7.7%)	8 (20.5%)	0.104
Coronary artery disease	2 (5.1%)	2 (5.1%)	>0.999
Donor AST (IU?L)	45 (9–483)	42 (13–294)	0.487
Donor ALT (IU/L)	40 (7–193)	32 (9–165)	0.171
Donor T-Bil (mg/dl)	0.8 (0.3–4.0)	0.8 (0.2–2.7)	0.756
Donor PT-INR	1.23 (0.9–2.0)	1.20 (0.9–2.8)	0.631
Donor Risk Index	1.5 (1.0–2.3)	1.3 (1.0–2.3)	0.385
Cold ischemia time (min)	429 (263–842)	463 (211–760)	0.355
Warm ischemia time (min)	53 (25–73)	51 (27–81)	0.656

Table 4.

Multivariate analysis for EAD in 458 primary LTs.

Pre-LT rifaximin < 28 days and longer CIT were identified independent risk factors for EAD.

Factors	Odds ratio	95% C.I. (lower-upper)	p value
Pre-LT rifaximin < 28 days	**2.096**	**1.298–3.382**	**0.002**
CIT (min)	**1.003**	**1.002–1.005**	**<0.001**
WIT (min)	1.012	0.998–1.027	0.096
Donor age (years)	1.016	0.995–1.038	0.135
Donor BMI (kg/m^2)	1.029	0.991–1.069	0.136
DRI (point)	0.858	0.392–1.879	0.701

Table 5.
Multivariate analysis for EAD in 230 patients with high MELD (MELD≥35)

Pre-LT rifaximin < 28 days and longer WIT were identified independent risk factors for EAD.

Factors	Odds ratio	95% C.I. (lower-upper)	p value
Pre-LT rifaximin < 28 days	**2.015**	**1.071–3.792**	**0.03**
WIT (min)	**1.052**	**1.026–1.079**	**<0.001**
Donor age (years)	1.014	0.992–1.035	0.21
Donor BMI (kg/m^2)	1.027	0.968–1.090	0.377