Microbial Functional Change is Linked with Clinical Outcomes after Capsular Fecal Transplant in Cirrhosis

Jasmohan S. Bajaj, …, Masoumeh Sikaroodi, Patrick M. Gillevet


Clinical Medicine In-Press Preview Hepatology

Background: Hepatic encephalopathy (HE) is associated with poor outcomes. A prior randomized, pilot trial demonstrated safety after oral capsular FMT in HE with favorable changes in microbial composition and cognition. However, microbial functional changes are unclear. Aim: Determine impact of FMT on gut-brain axis compared to placebo using microbial function based on bile acids (BA), inflammation (serum IL-6, lipopolysaccharide-binding protein, LBP), and EncephalApp. Methods: 20 cirrhotic patients were randomized 1:1 into receiving one-time FMT capsules from a donor enriched in Lachnospiraceae and Ruminococcaceae, or placebo capsules with 5-month follow-up for safety outcomes. Stool microbiota and BA, serum IL-6, BA and LBP, and EncephalApp were analyzed at baseline and 4-weeks post-FMT/placebo. Correlation networks between microbiota, BAs, EncephalApp, IL-6 and LBP were performed pre/post-FMT. Results: FMT-assigned participants had one HE recurrence and 2 unrelated infections. Six placebo-assigned participants developed negative outcomes. FMT, but not placebo, was associated with reduced serum IL-6 and LBP and improved EncephalApp. FMT-assigned participants demonstrated higher deconjugation and secondary BA formation in feces and serum compared to baseline. No change was seen in placebo. Correlation networks showed greater complexity post-FMT compared to baseline. Beneficial taxa such as Ruminococcaceae, Verrucomicrobiaceae […]

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Microbial Functional Change is Linked with Clinical Outcomes after Capsular Fecal Transplant in Cirrhosis

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Abbreviations: FMT: fecal microbiota transplant, HE: hepatic encephalopathy, BA: bile acid, MELD: model for end-stage liver disease, IND: investigational new drug, FDA: food and drug administration, QIIME2: quantitative insight into microbial ecology, LEfSe: linear discriminant analysis effect size, UDCA: ursodeoxycholic acid, NMR: nuclear magnetic resonance, PCA:
principal component analysis, PLS-DA: partial least squares-discriminant analysis, LBP: serum lipopolysaccharide binding protein

Keywords:
Randomized clinical trial; hepatic encephalopathy; bile acids; metabolomics; gut-brain axis

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Abstract

Background: Hepatic encephalopathy (HE) is associated with poor outcomes. A prior randomized, pilot trial demonstrated safety after oral capsular FMT in HE with favorable changes in microbial composition and cognition. However, microbial functional changes are unclear. Aim: Determine impact of FMT on gut-brain axis compared to placebo using microbial function based on bile acids (BA), inflammation (serum IL-6, lipopolysaccharide-binding protein, LBP), and EncephalApp.

Methods: 20 cirrhotic patients were randomized 1:1 into receiving one-time FMT capsules from a donor enriched in Lachnospiraceae and Ruminococcaceae, or placebo capsules with 5-month follow-up for safety outcomes. Stool microbiota and BA, serum IL-6, BA and LBP, and EncephalApp were analyzed at baseline and 4-weeks post-FMT/placebo. Correlation networks between microbiota, BAs, EncephalApp, IL-6 and LBP were performed pre/post-FMT.

Results: FMT-assigned participants had one HE recurrence and 2 unrelated infections. Six placebo-assigned participants developed negative outcomes. FMT, but not placebo, was associated with reduced serum IL-6 and LBP and improved EncephalApp. FMT-assigned participants demonstrated higher deconjugation and secondary BA formation in feces and serum compared to baseline. No change was seen in placebo. Correlation networks showed greater complexity post-FMT compared to baseline. Beneficial taxa such as Ruminococcaceae, Verrucomicrobiaceae and Lachnospiraceae were correlated with cognitive improvement and decrease in inflammation post-FMT. Fecal/serum secondary/primary ratios and PiCRUST secondary BA pathways did not increase in participants who developed poor outcomes.

Conclusions: Gut microbial function in cirrhosis is beneficially affected by capsular FMT with improved inflammation and cognition. Lower secondary BAs in FMT recipients could select for participants who develop negative outcomes.

Trial registration: www.clinicaltrials.gov NCT03152188
Funding: NCATS NIH R21TR002024 to JSB and NS, VA Merit Review Grant 2I0CX001076 to JSB, SDT-R is grateful to the United Kingdom National Institute for Health Research Biomedical Facility at Imperial College London for infrastructure support. The NMR Facility of the Centre for Biomolecular Spectroscopy was funded by British Heart Foundation, Wellcome Trust and King’s College London.
**Introduction:**

An altered gut-liver-brain axis underlies the pathogenesis of hepatic encephalopathy (HE) (1, 2). Therefore, beneficial alterations of the microbiota composition and function in HE is usually the mainstay of therapy (1). Treatments using laxatives such as lactulose and non-absorbable antibiotics such as rifaximin are traditionally used but in patients not responding to such therapies, other options are required (1). Fecal microbial transplant (FMT), using the oral capsular and enema route has the capability of changing the microbial milieu in several diseases (3, 4). In a prior randomized trial of patients with HE already on rifaximin and lactulose, FMT capsules were associated with engraftment of donor microbiota into the stool and mucosa, which resulted in improved cognitive function using OffTime+OnTime of the EncephalApp and decrease in overall hospitalizations compared to placebo (3, 4). In this study, we found that three FMT-assigned patients either had an infection or were hospitalized, while 6 placebo-assigned patients experienced similar complications. There was also a reduction in serum lipopolysaccharide binding protein (LBP) in FMT but not in placebo patients.

While there was compositional improvement, interaction of inflammation, bacterial translocation, and microbial function (bile acid, BA) with cognition needs to be evaluated (4). Gut microbiota can transform BAs by deconjugation, converting primary BAs to secondary & tertiary (oxo, sulfated, urso and iso-BA) forms, which have important functional consequences (5). Our aim was to determine the linkage between changes in microbiota after FMT with changes in serum and fecal BA moieties, inflammation, untargeted metabolic profiling of urine and serum, and cognition in patients with cirrhosis and HE, compared to placebo.
Results:

We enrolled 20 patients who were matched with respect to demographics, cirrhosis severity and cognitive testing (supplementary table 2, supplementary figure 1). All patients were on lactulose and rifaximin and the clinical course was characterized by 6 patients with serious adverse events in the placebo and 1 person in the FMT group (supplementary table 3). There were also three patients in the placebo and two in the FMT group with infections, which were considered unrelated by the Data Safety Monitoring Board. None of the clinical laboratory data significantly changed over time between and within groups (Supplementary table 4).

Microbiota analysis: As published before, there was an increase in relative abundance of Lachnospiraceae and Ruminococcaceae in the FMT group post-FMT compared to baseline, while there were no significant changes in the stool of the placebo group (4). There was also an increase in relative abundance of these taxa in the duodenal and sigmoid mucosa in the FMT group, indicating satisfactory engraftment.

Cognitive change: As published before, there was a significant improvement i.e. reduction in EncephalApp OffTime+OnTime in the FMT group (pre 277.8±123.5 vs 226.7±56.1 seconds, p=0.04 paired t-test), but not in placebo group (pre 318.9±181.0 vs post 308.9±169.5 p=0.47).

Inflammation: After FMT, but not placebo, there was a significant reduction in in serum IL-6 (Figure 1B) (4)

Metabolomic data: Excellent quality nuclear magnetic resonance (NMR) data were obtained from all the serum and urine samples. However, neither the serum nor urine NMR data sets showed differences pre-FMT/post-FMT, pre-placebo/post-placebo or post-FMT/post-placebo using multivariate analyses techniques (PCA, class separation 0.00 and PLS-DA, Q2<0.25). Detailed results are in the supplementary data.

Bile acid analysis:

Fecal and serum BA: We focused on three levels of microbial action on fecal BAs: Deconjugation, $7\alpha$ dehydroxylation and formation of tertiary BAs. At baseline, there were no
significant differences in the concentrations of fecal BAs (Table 1) as well the relative proportion of primary, secondary and tertiary BAs between placebo and FMT groups. Four weeks after FMT capsules, there was a statistically similar TBA between and within groups. Post-FMT there was an increase in deconjugation evidenced by a lower conjugated BA concentration. This was accompanied by a significant reduction in total primary BAs, increase in secondary BAs and secondary/primary BA ratio post-FMT (Figure 2A-E). There was a reduction prominent in cholic acid but both deoxycholic and lithocholic acid concentrations increased after FMT. No significant change in fecal tertiary BAs (iso, o xo or sulfated) were seen. This was accompanied by a similar pattern in serum, with lower primary and higher secondary BAs in the FMT, but not in the placebo group (Table 2, Figure 2F-G). Conjugation status did not change and both glyco- and tauro-conjugated primary BA moieties (chenodeoxycholic and cholic) reduced post-FMT. No changes in total BAs were seen in the serum nor were changes in the tertiary or conjugated BAs seen in the post-FMT compared to placebo groups.

**PiCRUST results:** Focusing on predicted functionality for secondary BA synthesis, we found a significantly higher expression post-FMT compared to baseline, while a trend towards lower expression was seen in the placebo group over the same period (Figures 4A-B).

**Correlation network analysis:**
There was a significant increase in cumulative distribution frequency of the correlations between microbiota, EncephalApp, bile acids, IL-6 and LBP post-FMT compared to the pre-FMT state (Figure 3A). The actual network differences are shown in Figure 3B. A high score on EncephalApp indicates poor performance. After FMT, Verrucomicrobiaceae were negatively associated with EncephalApp and IL-6 and positively with Ruminococcaceae. Baseline positive correlation of Enterococcaceae with EncephalApp and LBP disappeared after FMT. IL-6 showed new negative correlations with Clostridiales Incertae Sedis XIV post-FMT. Baseline negative Saccharibacteria correlations with EncephalApp and Lachnospiraceae were not seen
post-FMT. Fecal bile acid changes included positive linkage of conjugated deoxycholic acid with IL-6 and negative with EncephalApp at baseline, which disappeared post-FMT.

Association of microbial functional changes on outcomes:

Four FMT-assigned and seven placebo-assigned patients had low secondary/primary BA ratios in serum and feces at baseline. Of these, three FMT-assigned patients continued to not have an increase in the secondary/primary BA ratios compared to baseline even after FMT in the serum or feces, while in placebo group, this was seen in all six patients (Figures 2C-G). This corresponded to the PiCRUST secondary BA synthesis genes.

Of the three, one was admitted with HE within 30 days, and two had subsequent infections that were treated as an outpatient. All six of the placebo group that did not increase their secondary BAs developed HE or infections during the follow-up. Therefore, all 9 patients who developed outcomes during the follow-up did not demonstrate appreciable fecal or serum secondary/primary BA ratios. None of the other BA moieties, IL-6, LBP or untargeted metabolomics were associated with outcomes.
Discussion:

The data demonstrate that after oral capsular FMT performed as a randomized, placebo-controlled trial, there are distinct microbial functional changes in the FMT-assigned compared to the placebo-assigned group. Improvements in cognitive function were linked with changes in *Verrucomicrobiaceae*, IL-6 and *Ruminococcaceae* in oral capsular FMT. Microbial transformation of bile acids was associated with outcomes and may be relevant to monitor the success of the FMT.

Microbial perturbations are associated with the pathogenesis, and prognostication in HE and most therapies are focused on improving these alterations (1, 6). However, a major subset of patients continues to suffer cognitively and clinically despite the standard of care, which is lactulose and rifaximin in the US (7). The prevention of further deterioration is important in these patients. Two prior randomized clinical phase 1 trials have shown safety of the enema and oral capsular route in FMT in cirrhosis and recurrent HE compared to the comparator group (3, 4). The enema trial, performed after pre-FMT broad-spectrum antibiotics, was associated with improved cognition and lowered hospitalizations (3). However, functional changes in bile acid physiology, short-chain fatty acids and untargeted metabolomics in this trial largely demonstrated a restoration of pre-antibiotic baseline (8). In the cirrhosis population with limited ability to restore diversity, this is relevant, but questions regarding the role of FMT itself in altering microbial function in cirrhosis are still open.

Therefore, the analysis of microbial functional change after FMT in the oral capsular FMT trial, which was done without pre-intervention broad-spectrum antibiotics, was performed. We found changes in several aspects of microbial function and inflammation as a result of FMT in this study, which demonstrates a potential beneficial impact. There was a significant alteration in the bacterial bio-transformation of bile acids, which spanned both fecal and serum compartments. After oral capsular FMT, there was a greater deconjugation and 7α-dehydroxylation compared to baseline and to the placebo group, which resulted in higher secondary/primary fecal and serum
BA ratios. No changes in UDCA or tertiary BA moieties were seen. De-conjugation is the first step before 7α-dehydroxylation, it is usually not impaired in decompensated cirrhosis (5). On the other hand, patients with decompensated cirrhosis typically are not able to produce secondary BAs due to the relative reduction in Clostridial spp (5, 9). The microbiota in the donor was enriched in these taxa and likely resulted in the increases in secondary BA. These findings were also corroborated by the predicted functionality assay using PiCRUST. These particular set of changes demonstrate a step towards return to healthy microbial function, which have also been seen after successful FMT in patients with C. difficile (10). Indeed, all nine subjects who had adverse events such as recurrent HE and infections, had a continued lack of increase in secondary/primary BA ratios in the serum and feces. These included three FMT patients whose secondary BA profiles and whose corresponding PiCRUST predicted genes remained stagnant compared to pre-FMT. Secondary BAs are associated with protection from pathogenic organisms, indicate a healthy functional microbial diversity and could also indicate presence of taxa that make other compounds to reduce pathogenic microbiota (11, 12). The changes in BA bio-transformation in cirrhosis could have prognostic significance and could be a tool to ensure engraftment from a functional, rather than simply a compositional perspective.

The immuno-inflammatory milieu is also an important pathogenetic determinant of outcomes and cognitive impairment in cirrhosis (13). We found a reduction in serum LBP and IL-6 post-FMT but not after placebo, which demonstrate improvement in this milieu. Moreover, correlation network analyses showed a change in complexity and that beneficial microbial taxa such as Ruminococcaceae and Verrucomicrobiaceae were linked with the improved immuno-inflammatory milieu and with EncephalApp performance. Verrucomicrobiaceae, which includes Akkermansia muciniphila, are associated with decreased inflammation and strengthened intestinal barrier in patients with and without liver disease (14-17). In addition, the presence of these taxa was associated with lowered neuro-inflammation in mice colonized post-FMT from humans with cirrhosis (14). We chose the human donor enriched in Ruminococcaceae, which in
turn has taxa that can dehydroxylate primary to secondary bile acids and were associated with lowered IL-6 post-FMT. These correlations point towards a beneficial shift in the immuno-inflammatory milieu systemically and locally due to FMT that could result in improvement in cognitive performance.

It is intriguing that untargeted metabolomics of the serum and urine did not reflect changes overall in microbial or other metabolites either within or between groups. This contrasted with findings from the prior enema FMT trial, where the urinary metabolite changes, observed after both FMT and antibiotics, returned to baseline post-FMT (3). These differences could be due to the major changes influenced by broad-spectrum antibiotics that were likely not reflected by the capsular FMT. It is also likely that several changes brought on by FMT alone were more at the intestinal interface and focused on bacterial products such as LBP and bile acids rather than an overall change in metabolic milieu. Therefore, a more focused approach to metabolomics, rather than an untargeted one, or utilizing fecal metabolomics, could be important to tease out results based on FMT without antibiotics.

Our study is limited by relatively small number of patients who received oral capsular FMT and even smaller who developed infections or HE. Therefore, larger sample sizes are needed to further evaluate whether changes in BA profiles can predict outcomes definitively. All our subjects, regardless of oral capsular FMT or placebo, were on rifaximin and PPI, which can impact microbial function and could affect engraftment(18, 19). However, these therapies remained constant throughout the study and rifaximin is associated with an increase in fecal secondary BA concentrations(9). We also performed microbial functional analyses at week 4 rather than at the end of the study. However, engraftment and resultant bacterial function changes are more likely closer to the FMT rather than later. Future studies are needed in patients with cirrhosis not on proton pump inhibitors, lactulose or rifaximin. Given the recent reports of infection transmission with FMT from donors that were not checked with updated strict protocols, this remains an investigational therapy and further research is needed(20). The donor
in this study was negative for ESBL-producing *E.coli* and other known pathogens per the strict requirements of Openbiome(21).

We conclude that gut microbial function is beneficially affected by capsular FMT with amelioration of the systemic inflammatory milieu and cognitive improvement in cirrhosis and recurrent HE. The absence of secondary bile acids in FMT recipients could select for participants who develop poor outcomes on follow-up. Larger studies are needed to determine the ability of early microbial functional change to prognosticate longer-term outcomes after FMT in cirrhosis and HE.
Methods:

Randomized placebo-controlled clinical trial: As previously published, we enrolled patients with cirrhosis with recurrent HE already on lactulose and rifaximin, all of whom were also on proton pump inhibitors, into a clinical trial under FDA IND(Figure 1A), which was registered on www.clinicaltrials.gov NCT03152188 (4). Patients were randomized 1:1 into receiving 15 FMT capsules all at one occasion vs identical placebo. FMT was from a single donor enriched in Lachnospiraceae and Ruminococcaceae, selected through machine learning. Capsules were made The details of the trial design and eligibility criteria and methods are in the supplementary data (Supplementary table 1 and text). We administered 15 capsules of FMT vs placebo once to each participant. The 15 capsules contain 4.125 grams of stool, which are created using wet preparation of stool without lyophilization.

Endpoints of the trial: All hospitalizations and infections, serious adverse events related to FMT, cognitive function using psychometric hepatic encephalopathy score (PHES) and EncephalApp Stroop, and microbial composition at the mucosal and fecal levels were analyzed (4). This was compared between and within groups at baseline and 4 weeks post-intervention and subjects were followed for 5 months. All groups provided serum, urine and stool at the screening visit, study baseline visit, safety visit (1-2 weeks after placebo or FMT capsules) and at 4 weeks after placebo or FMT capsule.

Microbial composition and function:

Microbial composition: We used published techniques to evaluate 16SrRNA sequencing from stool and mucosal biopsies from the sigmoid and duodenum. Diversity and relative abundances were compared between and within groups using Shannon indices and QIIME2 (quantitative insight into microbial ecology).

Microbial function:

Bile acid analysis: Serum and fecal BA analysis were performed using published LC/MS techniques (22). Details and specific moieties that were determined are in the supplementary
data. We determined the total BA, primary BA, secondary BA, conjugated BA and tertiary BAs at baseline and study end and within groups. Tertiary BAs included ursodeoxycholic acid (UDCA), oxo and iso-forms of BAs. Secondary/primary fecal BA ratios were also calculated. PiCRUST (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States)(23) was performed focusing on secondary BA synthesis pathway in patients before/after FMT and before/after placebo with respect to stool microbiota.

Untargeted metabolomics: Urine and serum samples were prepared for NMR analyses using standardized protocols(24). NMR data were acquired at the Centre for Biomolecular Spectroscopy, King’s College London using a Bruker 600MHz (AVANCE NEO) NMR spectrometer with a $^1$H/$^{13}$C/$^{15}$N TCI Prodigy (nitrogen-cooled) probe (Bruker Corporation, Billerica, USA). Pulse-collect (urine, serum) and spin-echo (serum) NMR data sets were acquired using PURGE water suppression(25), PROJECT spin-echo and J-resolved NMR sequences. NMR data from pre- and post-FMT, pre- and post-placebo and post-FMT and post-placebo groups were compared using principal component analyses (PCA, KnowItAll® Informatics, Metabolomics Edition v17.0, BioRad, Berkeley, USA) and partial least squares-discriminant analysis (PLS-DA) (MetaboAnalyst 4.0, McGill University, Canada)(26). Detailed methods are in the supplementary data.

Systemic inflammation: Serum IL-6 and serum lipopolysaccharide binding protein (LBP) were analyzed using published ELISA techniques (Supplementary methods).

Correlation networks using published R techniques were ultimately created to determine linkages between gut microbiota, liver disease severity (MELD score), cognitive function, systemic inflammation and serum and fecal BAs for the group receiving FMT(27). Pre-FMT baseline was compared to post-FMT day 20 assessments using R and the specific correlation network differences were then highlighted. Cumulative distribution frequencies were compared between groups. We specifically focused on EncephalApp OffTime+OnTime performance, IL-6,
LBP and the two taxa for which the donor was selected, Lachnospiraceae and Ruminococcaceae.

**Effect on outcomes:** Lastly, 3 of the FMT patients either developed an infection or were hospitalized and six of the placebo patients developed these outcomes. We analyzed the changes in microbial function in these patients compared to the remaining FMT and placebo recipients.

**Statistics:**

We performed Wilcoxon matched pairs analysis pre/post FMT and pre/post placebo as well as Mann-Whitney U test for medians as appropriate. Multiple comparisons analyses as appropriate were performed using Dunn’s multiple comparisons test (Tables 1 and 2). Graphpad Prism and Minitab software was used for analysis and figures. Cytoscape was used to visualize correlation network differences, which were calculated using R software.

**Study approval:** This study was approved by the IRB at Virginia Commonwealth University and McGuire VA Medical Centers. All participants provided written informed consent for the study.
Author Contributions:
JSB and NS conceptualized and obtained funding, NS, MLK and MH were responsible for inflammatory analyses, HN, HT, GK, MWP and PH were involved in bile acid analysis, MW, AF, MF, HL, SM, PP, MSS, JSB, CA, EAG were involved in the clinical trial and sample collection and processing, IJC, STR, LA, RW, AA, AL were involved in untargeted metabolomics, while MS and PMG were involved in microbiota analysis and bio-informatics.

Acknowledgements: NCATS NIH R21TR002024 to JSB and NS, VA Merit Review Grant 2I0CX001076 to JSB, SDT-R is grateful to the United Kingdom National Institute for Health Research Biomedical Facility at Imperial College London for infrastructure support. The NMR Facility of the Centre for Biomolecular Spectroscopy was funded by British Heart Foundation, Wellcome Trust and King’s College London.
References:


1A: Schema of the first 4 weeks with red arrows indicating the time of sample collection

1B: Serum IL-6 in pg/mL change within placebo group was not significant, 1C: Serum IL-6 in the FMT group showed significant reduction compared to baseline. P values on Wilcoxon matched pair tests are shown atop each figure, FMT: Fecal microbiota transplant
Figure 2:
Stool BA composition within groups:
There was a significant reduction in conjugated and primary BAs and an increase in secondary BAs post-FMT compared to baseline (A).
No significant change in these moieties was seen in the placebo group (B)
Blue=baseline, Red=post-intervention
*p<0.05 on Wilcoxon matched pairs test as appropriate
Data presented as ug/g of dried stool
Figures 3A-B:
Stool Secondary/Primary BA ratio:
Individual values showing similar values in placebo (3A) and increase in all but three in FMT (3B)

Figures 3C-D:
Serum Secondary/Primary BA ratio:
Individual values showing similar values in placebo (3C) and increase in all but three in FMT (3D)

All comparisons were done using Wilcoxon matched pairs test as appropriate. 10 samples in each group and each timepoint was included.
Figures 4 A-B:
Stool PiCRUST pathway
Activity pertaining to secondary bile acid synthesis

All comparisons were done using Wilcoxon matched pairs test as appropriate.
Three patients in the FMT group had similar or decreased secondary BA analysis compared to the rest.

Most patients in the placebo group had a decrease in secondary BA synthesis pathway genes over time, which had a trend towards statistical significance.

10 samples in each group and each timepoint was included
4A: Cumulative distribution frequency post-FMT was higher than pre-FMT in the correlation networks, Orange: Post-FMT correlation network, Blue: Pre-FMT correlation network
5B: Pre vs post-FMT correlation network differences showed changes centered around EncephalApp and serum IL-6. Favorable linkages between EncephalApp (high score=poor) and Verrucomicrobiaceae, Ruminococcaceae and IL-6 were seen after FMT, which was a change from the pre-FMT baseline. Purple lines: Positive after FMT; none at baseline, Green lines: Negative after FMT; none at baseline, Black lines: Positive at baseline; none after FMT, Orange lines: Negative at baseline; none after FMT
Table 1: Stool Bile Acid Concentrations

<table>
<thead>
<tr>
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<th>Placebo</th>
<th>Fecal Microbial Transplant</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End</td>
</tr>
<tr>
<td>Total BAs</td>
<td>2.67 (4.38)</td>
<td>2.6 (8.26)</td>
</tr>
<tr>
<td>Total Conjugated</td>
<td>0.39 (0.89)</td>
<td>0.29 (0.55)</td>
</tr>
<tr>
<td>Total Primary</td>
<td>1.65 (1.35)</td>
<td>1.46 (2.41)</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>0.38 (1.0)</td>
<td>0.21 (1.04)</td>
</tr>
<tr>
<td>Chenodeoxycholic acid</td>
<td>0.38 (0.75)</td>
<td>0.27 (0.43)</td>
</tr>
<tr>
<td>Total Secondary BAs</td>
<td>1.25 (4.2)</td>
<td>0.80 (7.2)</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>0.10 (1.48)</td>
<td>0.10 (2.71)</td>
</tr>
<tr>
<td>Lithocholic acid</td>
<td>0.18 (1.13)</td>
<td>0.10 (0.95)</td>
</tr>
<tr>
<td>Secondary/Primary BA</td>
<td>0.18 (7.03)</td>
<td>0.53 (5.40)</td>
</tr>
<tr>
<td>Total Tertiary</td>
<td>0.62 (1.47)</td>
<td>0.90 (2.55)</td>
</tr>
<tr>
<td>Total Oxo BAs</td>
<td>0.26 (0.50)</td>
<td>0.28 (0.62)</td>
</tr>
<tr>
<td>Total Iso BAs</td>
<td>0.10 (0.57)</td>
<td>0.10 (1.01)</td>
</tr>
<tr>
<td>Total Sulfated</td>
<td>0.01 (0.42)</td>
<td>0.01 (0.62)</td>
</tr>
</tbody>
</table>

Data are presented as median (IQR), *p<0.05 between groups on Mann-Whitney test, ‡p<0.05 on Wilcoxon signed rank matched pairs tests, BA: bile acid,
<table>
<thead>
<tr>
<th>Median serum</th>
<th>Placebo</th>
<th>Fecal Microbial Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End</td>
</tr>
<tr>
<td>Total BAs</td>
<td>50.3 (53.7)</td>
<td>54.9 (41.1)</td>
</tr>
<tr>
<td>Total Primary</td>
<td>50.3 (57.0)</td>
<td>54.9 (39.8)</td>
</tr>
</tbody>
</table>

**Individual Primary BAs**

- **Glyco-chenodeoxycholic acid**
  - Placebo: 19.0 (17.8) / 18.0 (22.6)
  - FMT: 18.9 (28.5) / 14.6 (28.6)‡

- **Tauro-chenodeoxycholic acid**
  - Placebo: 15.2 (20.5) / 13.0 (16.1)
  - FMT: 14.7 (20.4) / 10.0 (16.6)‡

- **Glyco-cholic acid**
  - Placebo: 5.7 (4.8) / 5.0 (4.9)
  - FMT: 7.3 (8.6) / 4.1 (6.1)‡

- **Tauro-cholic acid**
  - Placebo: 4.6 (6.4) / 4.1 (4.1)
  - FMT: 5.1 (6.7) / 2.9 (6.4)‡

- **Total Deconjugated**
  - Placebo: 2.5 (4.3) / 1.8 (4.5)
  - FMT: 2.1 (3.9) / 2.0 (3.1)

- **Cholic acid**
  - Placebo: 0.23 (0.52) / 0.15 (0.57)
  - FMT: 0.17 (0.43) / 0.12 (0.18)

- **Chenodeoxycholic acid**
  - Placebo: 1.1 (4.0) / 1.0 (3.2)
  - FMT: 0.9 (2.8) / 0.6 (1.4)

- **Total Secondary**
  - Placebo: 0.02 (1.06) / 0.03 (1.36)
  - FMT: 0.03 (1.9) / 1.23 (2.5)‡

- **Glyco-Deoxycholic acid**
  - Placebo: 0.0 (0.17) / 0.0 (0.61)
  - FMT: 0.05 (0.66) / 0.25 (1.1)

- **Tauro-Deoxycholic acid**
  - Placebo: 0.0 (0.04) / 0.0 (0.06)
  - FMT: 0.10 (0.56) / 0.14 (0.52)

- **Deoxycholic acid**
  - Placebo: 0.0 (0.53) / 0.0 (0.67)
  - FMT: 0.40 (0.75) / 0.20 (0.47)

- **Secondary/ Primary**
  - Placebo: 0.000 (0.002) / 0.000 (0.000)
  - FMT: 0.002 (0.005) / 0.006 (0.02)‡

**UDCA moieties**

- **Glyco-UDCA**
  - Placebo: 0.15 (0.61) / 0.11 (0.65)
  - FMT: 0.62 (1.1) / 0.38 (1.4)

- **Tauro-UDCA**
  - Placebo: 0.07 (0.40) / 0.07 (0.25)
  - FMT: 0.19 (0.48) / 0.07 (0.48)

- **UDCA**
  - Placebo: 0.03 (0.06) / 0.06 (0.06)
  - FMT: 0.07 (0.22) / 0.02 (0.30)

Data are presented as median (IQR), *p<0.05 between groups on Mann-Whitney test, ‡p<0.05 on Wilcoxon signed rank matched pairs tests, BA: bile acid, UDCA: urso-deoxycholic acid