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Transjugular intrahepatic Porto-systemic shunt positively influences the composition and metabolic functions of the gut microbiota in cirrhotic patients

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ABSTRACT

Background & aims: Cirrhosis and its complications may affect gut microbiota (GM) composition. Transjugular intrahepatic portosystemic shunt (TIPS) represents the most effective treatment for portal hypertension (PH). We aimed to evaluate whether TIPS placement modifies GM composition and metabolic function.

Methods: A compositional and functional GM analysis was prospectively performed in 13 cirrhotic patients receiving TIPS. Patients receiving systemic or non-absorbable antibiotics for any indications were excluded. Fecal samples were collected before and three months after TIPS. GM was analyzed by 16S ribosomal RNA sequencing. Small- and medium-chain fatty acids (SCFAs and MCFAs, respectively) were measured by gas chromatography/mass spectrometry.

Results: TIPS placement resulted in a mean 48% reduction in portal-caval pressure gradient. No recurrence of PH related complications was observed. After TIPS, increased levels of *Flavonifractor* spp. ($p = 0.049$), and decreased levels of Clostridiaceae ($p = 0.024$), these latter linked to abdominal infections in cirrhotic patients, were observed. No differences were found in the SCFAs signature while analysis of MCFA profiles showed a decreased abundance of pro-inflammatory isohexanoic ($p<0.01$), 2-ethylhexanoic ($p<0.01$) and octanoic acids ($p<0.01$) after TIPS.

Conclusion: Correction of PH following TIPS results in modifications of GM composition which could be potentially beneficial and reduces the levels of fecal pro-inflammatory MCFAs.

List of abbreviations: TIPS, transjugular intrahepatic portosystemic shunt; PH, portal hypertension; GM, gut microbiota; SCFAs, small-chain fatty acids; MCFAs, middle-chain fatty acids; HE, hepatic encephalopathy; RA, refractory ascites; MELD, Model for end-stage liver disease; PSPG, porto-systemic pressure gradient; OUT, operational taxonomic unit; AUD, alcohol use disorder; PCoA, Principal Coordinate Analysis; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis (NASH).

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1. Introduction

Transjugular intrahepatic portosystemic shunt (TIPS) represents an effective treatment option for severe complications of portal hypertension (PH) in cirrhotic patients [1]. Recent advances in the understanding of the pathogenesis of advanced cirrhosis and its complications indicate that inflammation and host-microbiota interactions play a relevant role in cirrhosis decompensation [2]. Gut microbiota (GM) is the largest collection of commensal microorganisms in the human body, engaged in reciprocal cellular and molecular interactions with the liver [3]. This gut-liver axis involves several bacterial end products, such as short-chain fatty acids (SCFAs), which provide energy substrates for colonocytes and mitigate intestinal inflammation [4], and medium-chain fatty acids (MCFAs) which favor a switch towards the production of pro-inflammatory cytokines [5]. Consequently, perturbation of the GM profile may contribute to the development of complications of cirrhosis [6].

Cirrhosis and PH affect GM composition and increase translocation, which in turn increases portal pressure, creating a vicious cycle [7]. Specifically, PH increases gut permeability, facilitating systemic spillover of pathogens, metabolites or bacterial structural components, resulting in activation of the inflammasome system and of hepatic stellate cells, favoring the worsening of inflammation and fibrosis [7]. PH-associated alterations of the GM also predispose to spontaneous bacterial peritonitis and hepatic encephalopathy (HE), and have an impact on systemic hemodynamics, contributing to a hyper-dynamic circulatory state [7].

Based on the strong association between PH, inflammation, and GM profile, this study was undertaken to evaluate the changes in both the GM composition and function (fecal SCFA and MCFA profiles) following TIPS in a group of carefully selected patients with cirrhosis and complications of portal hypertension.

2. Patients and methods

2.1. Patients

Thirteen consecutive patients with cirrhosis of any etiology receiving TIPS for refractory ascites (RA) or for secondary prophylaxis of variceal bleeding were prospectively enrolled between June 2019 and June 2020. Inclusion criteria were: a) diagnosis of cirrhosis according to clinical history, histology or imaging; b) prevention of recurrence of variceal bleeding and/or RA as indications to TIPS; c) age >18 and <75 years. Exclusion criteria were: a) emergency TIPS placed in the setting of acute variceal bleeding as preemptive or rescue TIPS; b) non-cirrhotic portal hypertension; c) absolute contraindications to TIPS [8]; d) hepatocellular carcinoma or other malignancies; e) antibiotic prophylaxis or treatment including the management of HE (graded according to international guidelines [9]) and administration of prebiotics, probiotics or proton pump inhibitors, at the time of TIPS placement or within 3 month from the procedure; f) gastrointestinal surgery or surgical shunting before enrolment; g) previous history of HE; h) stent graft dysfunction or development of overt HE during post-operative review; i) pregnancy or lactation; j) intestinal colonization with multi drug resistant organisms; k) presence of comorbidities *a priori* affecting the GM composition (e.g. inflammatory bowel disease, celiac disease, severe chronic heart failure, severe obesity and l) unavailable stool samples.

Recorded patient information at the time of TIPS placement included demographic and clinical data (retrieved from an Italian survey on TIPS procedure, RiTIPS), etiology of cirrhosis, biochemistry (serum bilirubin, albumin, creatinine, sodium, INR, platelet count, C-reactive protein), Child-Pugh score, Model for end-stage liver disease (MELD) and MELD-Na score, indication to TIPS placement, porto-systemic pressure gradient (PSPG) before and after TIPS placement, and TIPS dilation diameter. All patients underwent scheduled control visits in a dedicated outpatient clinic including physical examination and biochemistry evaluation at 3 months from TIPS, unless TIPS dysfunction or other complications, including HE, were observed. At each visit, all patients were carefully assessed for overt HE by physical examination.

Doppler ultrasonography of TIPS was performed 1 weeks and 3 months after TIPS unless TIPS dysfunction was suspected. Fecal samples were collected 1–3 days before and 3 months (range 81–102 days) after TIPS and were stored at −80 °C within 2 h after sampling. The TIPS procedure was performed as previously described [10].

2.2. Gut microbiota characterization

Total DNA was extracted using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany) from frozen (−80 °C) stool samples, according to the manufacturer's instructions. Briefly, 0.25 g of stool samples were added to a bead beating tube and homogenized with TissueLyser LT (Qiagen, Hilden, Germany) for 5 min at 50 Hz. Afterwards, DNA was captured on a silica membrane in a spin column format, washed and eluted. The quality and quantity of extracted DNA was assessed with the Qubit Fluorometer (Thermo Fisher Scientific) and then it was frozen at −20 °C. Subsequently, genomic DNA samples were sent to IGA Technology Services (Udine, Italy) where amplicons of the variable V3–V4 region of the bacterial 16 s rRNA gene were sequenced in paired-end (2 × 300 cycles) on the Illumina MySeq platform, according to the Illumina 16S Metagenomic Sequencing Library Preparation protocol. Lastly, the raw data were processed following the software pipeline MICCA (MICrobial Community Analysis) as we previously described [11]. Briefly, paired end reads were assembled maintaining a minimum overlap of 20 bp and an edit distance in the maximum overlap of 2 bp. Next, the sequences were cut to remove the primers and all the reads having a length lower than 350 bp and with an error rate higher than or equal to 0.5 were removed. Cleaned reads were merged into a single file and transformed into a FASTA file. The OTUs were generated by setting a 97% identity and performing an automatic removal of chimeras and so the longest sequence of each OTU was used for the taxonomic assignment, i.e. using the RDP Bayesian classifier that is able to obtain classification and confidence for taxonomic ranks up to genus.

2.3. Analysis of fecal SCFAs and MCFAs by gas chromatography-mass spectrometry

The qualitative and quantitative evaluation of fecal SCFAs and MCFAs was performed by Agilent GC–MS system equipped with a 5971 single quadrupole mass spectrometer, 5890 gas-chromatograph and 7673 autosampler, using our previously described GC–MS method [12]. Briefly, just before the analysis, stool samples were thawed and 0.25 mM sodium bicarbonate solution (1:1 w/v) was added to a 1.5 mL centrifuge tube. Then, the obtained suspension was sonicated for 5 min, centrifuged at 5000 rpm for 10 min and then the supernatant was collected. The SCFAs were finally extracted as follow: an aliquot of 100 µl of sample solution (corresponding to 0.1 mg of stool sample) was added of 50 µL of internal standards mixture, 1 mL of tert–butyl methyl ether and 50 µL of HCl 6 M + 0.5 M NaCl solution in 1.5 mL centrifuge tube. Subsequently, each tube was shaken in a vortex apparatus for 2 min, centrifuged at 10,000 rpm for 5 min, and finally the solvent layer was transferred to an autosampler vial and processed three times.

2.4. Statistical analysis

Continuous variables are presented as median and interquartile range, and categorical variables as number and percentages. Shapiro-Wilk test was used to assess normality of distribution. Continuous variables were compared using a two-tailed paired *t*-test or Wilcoxon test, whereas the association between categorical data was determined using chi-square or Fisher's exact test as appropriate. A two-sided p value <0.05 was defined to be considered statistically significant.

Statistical analyses on the bacterial communities were performed in R (R Core Team, 2014) with the help of the packages phyloseq 1.26.1 [13], DESeq2 1.22.2 [14] and other packages satisfying their dependencies, in particular, vegan 2.5–5. For the cluster analysis (complete clustering on Euclidean distance) of the entire community, the operational taxonomic unit (OTU) table was first normalized using the total OTU counts of each sample and then adjusted using square root transformation. Rarefaction analysis on OTUs was performed using the function rarecurve (step 50 reads) [15], further processed to highlight saturated samples (arbitrarily defined as saturated samples with a final slope in the rarefaction curve with an increment in OTU number per read $<$ 1e-5).

Shannon, Chao and Evenness indices were used to estimate bacterial diversity in each sample using the function estimate_richness from phyloseq. The evenness index was calculated using the formula $E = S/\log(R)$, where S is the Shannon diversity index and R is the number of OTUs in the sample.

Differences in all indices between pre- and post-TIPS samples were tested using a paired Wilcoxon signed-rank test. The differential analysis of abundance at the OTUs as well as at different taxonomic ranks (created using the tax_glom function in phyloseq) was performed with DESeq2 using a two-group blocked by patient design in order to perform a paired test.

The software GraphPad Prism (v.5) was used for the statistical analysis of fecal SCFAs and MCFAs. Pairwise differences between groups were assessed using Wilcoxon rank-sum test and P-values less than 0.05 were considered statically significant.

2.5. Ethical issues

The present study was performed in accordance with the ethical standards defined in the 1964 Declaration of Helsinki and its later amendments and it was approved by the local Ethics Committee (Comitato Etico Area Vasta Centro, approval number: 20117_bio).

3. Results

3.1. Patients' characteristics

The baseline characteristics of the 13 patients enrolled are reported in Table 1. Median age was 58 years (interquartile range 13.2). The most common cause of liver disease was alcohol use disorder (AUD). All patients affected by AUD had stopped alcohol consumption at least 3 months before TIPS and none of the patients relapsed within 3 months after TIPS. Importantly, all patients but one were abstinent for at least 5 months before TIPS procedure, ranging from 5 to 23 months. Most of patients were in Child-Pugh class B, and the mean basal MELD was 10±2. The leading indication to TIPS was RA (61.5% of cases).

The modification of liver function indexes and the variations of C-reactive protein values from baseline to three months after TIPS are reported in the Supplementary Table 1.

Table 1
Characteristics of the patients enrolled in the study.

Variable	Median (IQR) or n (%)
Age (years)	58 (13.2)
Male gender	11 (84.62)
Etiology	
Alcohol	6 (46.15)
Cryptogenic	3 (23.08)
NASH	2 (15.38)
Viral	2 (15.38)
Child-Turcotte-Pugh class	
A	4 (30.76)
B	9 (69.23)
MELD score	11 (3)
MELD-Na score	11 (2,5)
TIPS indication	
Refractory ascites	8 (61.5)
PH bleeding prophylaxis	5 (38.5)

IQR, interquartile range; NASH, non-alcoholic steatohepatitis; MELD, model for end-stage liver disease; TIPS, trans-jugular intrahepatic porto-systemic shunt; PH, portal hypertension.

Table 2
Hemodynamic data.

Pts.	Pre-TIPS (mm Hg)			Post-TIPS (mm Hg)		
	PSPG	PP	ICVP	PSPG	PP	ICVP
1	26,5	32	5,5	14	24	10
2	32,8	38	5,2	5,5	15,3	9,8
3	32	37	5	5	13	8
4	22	29	7	4	12	8
5	25	35	10	10,4	22,2	11,8
6	19,7	30,7	11	12,2	24	11,8
7	20	29	9	8.5	20,9	12,4
8	17	25	8	8,7	18,4	9,7
9	25,6	31,4	5,8	13,3	21,6	8,3
10	24	40	16	15,5	35	19,5
11	19	24	5	10,5	20	9,5
12	16	26,3	10,3	9	22,8	13,8
13	20,5	33,5	13	10,5	29	18,5

PP, portal pressure; ICVP, inferior cava vein pressure; PSPG, porto-systemic pressure gradient; TIPS, trans-jugular intra-hepatic porto-systemic shunt.

3.2. Procedure and clinical outcomes

All patients underwent TIPS placement using Viatorr CX covered stent grafts (Gore, Flagstaff, AZ) deployed at 6 mm (balloon dilatation). In 7 out of 13 enrolled patients, in whom a six-month CT scan was available, intra-parenchymal dilation diameter of the device was substantially maintained, and maximal self-expansion was 1.4 mm. Hemodynamic data recorded during TIPS implantation are reported in Table 2. TIPS placement resulted in a marked and significant drop in PSPG ($p = 0.0005$) with a mean reduction of 48%. In all patients, the procedure was effective in controlling the complication of PH representing the indication to TIPS. MELD score was slightly, but significantly increased after TIPS (from 10±2 to 12±3, $p = 0.026$).

No patients experienced TIPS dysfunction or other complications. All patients were on traditional Mediterranean diet at baseline and during the follow-up. No patient was frankly sarcopenic, and therefore no protein dietary supplement was recommended.

3.3. Gut microbiota composition before and after TIPS

To compare the gut microbiota composition before and after TIPS, we firstly evaluated the alpha diversity of samples (Fig. 1). No significant differences were found with any of the three indexes used, namely Chao, Shannon and Evenness.

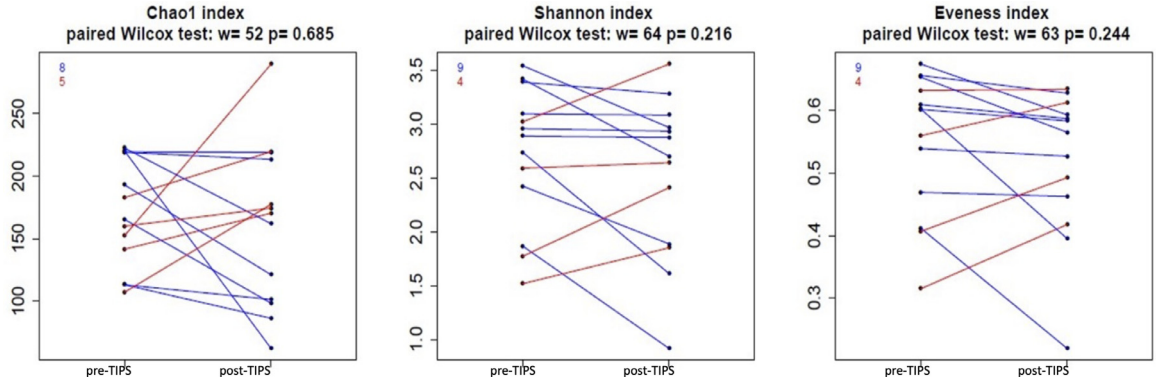

Fig. 1. Boxplots showing alpha diversity indices (Chao1 index, Shannon index, Evenness) in patients pre- and post-TIPS. Numbers in the top-left corner represent counts of decreased (blue) and increased (red) measurements for paired samples. Statistical differences were assessed using Wilcoxon signed-rank test and p-values less than 0.05 were considered statistically significant.

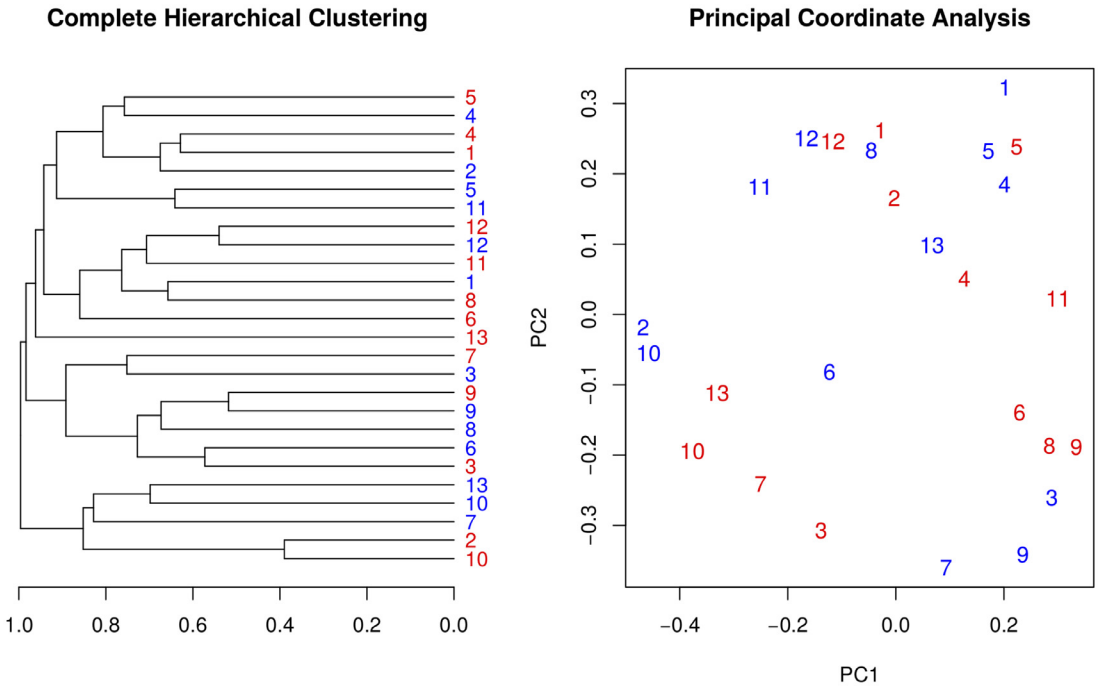

Fig. 2. Cluster analysis **(A)** and Principal Coordinate Analysis **(B)** of pre- and post-TIPS samples (red=pre-TIPS, blue=post-TIPS).

To explore the similarity of patients' gut microbiota abundance profiles and to study the paired nature of sampling (pre-TIPS vs. post-TIPS), we performed a cluster analysis and a Principal Coordinate Analysis (PCoA) on normalized OTU counts. No distinct groups were reported by hierarchical clustering, but we found that 8 out of the 13 patients showed a considerable modification of their gut microbiota composition after TIPS (Fig. 2A), as confirmed also by PCoA (Fig. 2B).

We next performed a paired comparison of the abundances of single microbial ranks before and after TIPS placement. A significant increase of the order Bacteroidales and a reduction in Actinomycetales and Bacillales were observed after TIPS (Fig. 3A). Analysis of the family taxonomic rank (Fig. 3B) showed that TIPS resulted in a higher abundance of Bacteroidaceae and Enterobacteriaceae, and decreased levels of Erysipelotrichaceae, Coriobacteriaceae, Peptostreptococcaceae, Actinomycetaceae and Clostridiaceae. At the genus level, after TIPS placement increased levels of *Bacteroides* spp., *Flavonifractor* spp. and *Escherichia/Shigella* spp., and lower abundance of *Lachnospiraceae incerta sedis* spp., *Dorea* spp., *Anaerostipes* spp., *Gemmiger* spp., *Collinsella* spp., *Gemella* spp., *Romboutsia* spp., *Clostridium sensu stricto* spp., *Actinomyces* spp. and *Atopobium* spp. were found (Fig. 3C).

3.4. Evaluation of fecal SCFAs and MCFAs profiles

To establish whether the different composition of gut microbiota after TIPS resulted in an actual modification of metabolic products released by bacteria, we evaluated the levels of fecal SCFAs (acetic, propionic, butyric, 2-methylbutyric, isobutyric, isovaleric and valeric acids) and MCFAs (hexanoic, isohexanoic, 2-ethylhexanoic, heptanoic, octanoic, nonanoic, decanoic and dodecanoic acids) in feces collected before and after TIPS. TIPS placement resulted in changes in MCFAs, with significantly decreased levels of isohexanoic, 2-ethylhexanoic and octanoic acids (Fig. 4). Conversely, no significant differences in fecal SCFAs levels were reported comparing samples collected before and after TIPS. Even when evaluated both the ratio between SCFAs and MCFAs, no significant differences were found.

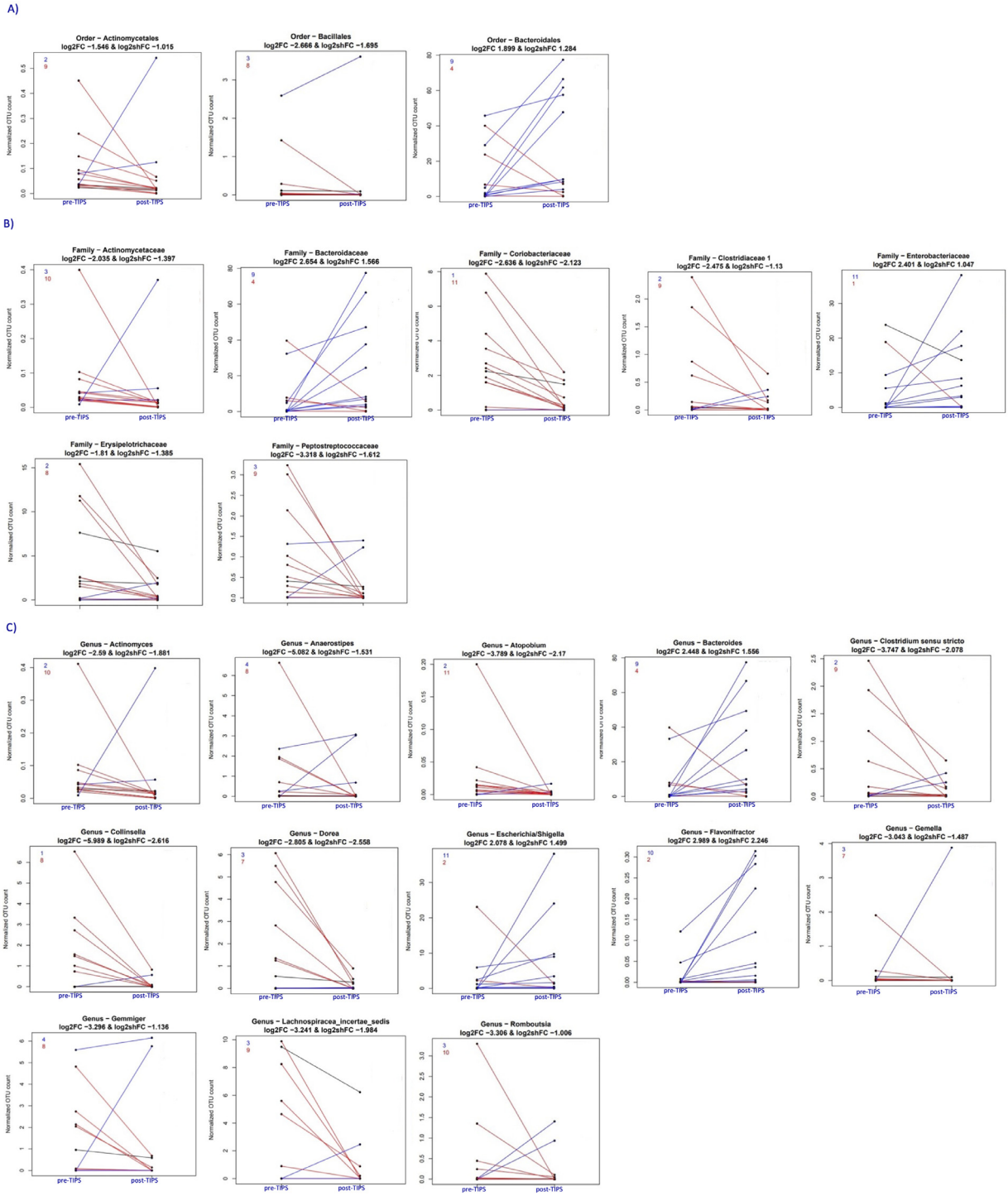

Fig. 3. Segment plots showing significantly different taxa between pre- and post-TIPS groups. Lines link paired samples and red or blue colors respectively highlight the decrease or increase of normalized abundance at order (A), family (B) and genus (C) level. Numbers in the top-left corner represent counts of decreased (red) and increased (blue) measurement for paired samples. Plot titles report both Log2 fold change and shrinked Log2 fold change (according to the DESeq2 function lfcShrink) between pre- and post-TIPS groups.

4. Discussion

Accumulating evidence indicates the role of the composition of GM and the resulting metabolic factors released by microbes in the pathogenesis of different forms of liver diseases and in cirrhosis [16]. Nonetheless, the complex interplay between the microbiota and pathophysiologic changes that accompany liver diseases, and especially cirrhosis, have only been marginally explored. In this study, we investigated the effects of correction of portal hypertension, a major pathogenic factor leading to the complications of cirrhosis, on GM composition and fecal concentration of SCFAs and MCFAs. To this aim, we analyzed patients before and after placement of a TIPS, which resulted in a marked, and overall significant, drop in portal pressure. While alpha diversity was not changed comparing samples collected before and after TIPS, we observed relevant modifications of the abundance of different

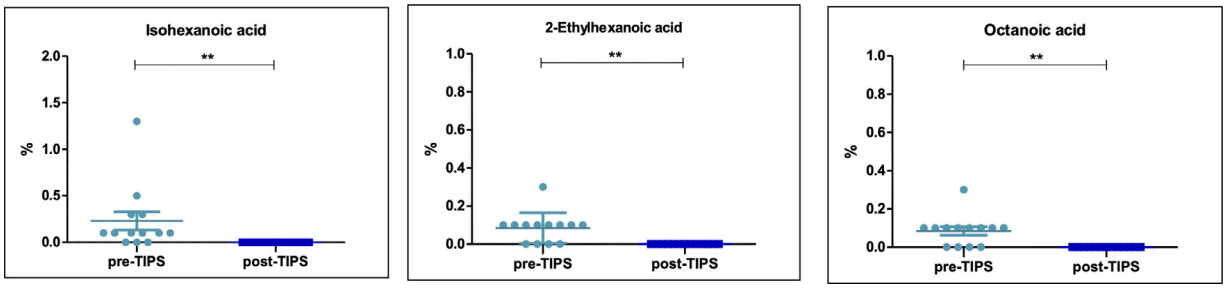

Fig. 4. Scatter plots representing the significant differences of fecal MCFAs in pre- and post-TIPS patients. Analysis was assessed using the paired Wilcoxon signed-rank test and p-values less than 0.05 were considered statistically significant. The asterisks (*) represent p-values, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

bacterial genera and species after reduction of portal pressure. In particular, we detected an increased abundance of *Flavonifractor spp*., which are considered representative of a favorable microbial environment, and the decline of other species associated with an unfavorable microbiota, such as *Clostridiaceae* members. Evidence for a beneficial role of *Flavonifractor* spp. derives mostly from the metabolic field and from studies in patients with non-alcoholic fatty liver disease (NAFLD) [17]. Jiang et al. [18] compared the gut microbiota composition in 53 NAFLD patients observing lower levels of both *Oscillibacter* spp. and *Flavonifractor spp,* compared to 32 healthy subjects. The reduction of these species in NAFLD may contribute to explain the presence of chronic low-grade inflammation [19]. In a recent study, increased levels of the genus *Flavonifractor* have been reported in cirrhosis after TIPS placement, in patients who did not experience HE [20]. This observation underscores the favorable changes induced by TIPS placement on gut ecology. Along these lines, reduced abundance of the genus *Flavonifractor* has been found in patients with cirrhosis [21], while it was higher in patients treated with L-ornithine L-aspartate (LOLA), which exerts beneficial effects in patients with HE [22]. Similarly, lower abundance of *Lachnospiraceae incertae sedis, Collinsella* and *Atopobium*, which are increased in patients affected by NAFLD [23], non-alcoholic steatohepatitis (NASH) [24], and cirrhosis [25], and reduced levels of *Gemella* spp., elevated in patients with primary biliary cirrhosis and correlated with systemic inflammation [25], was found following TIPS. Of note, an increase in Enterobacteriaceae, usually less represented in healthy subjects, was observed after TIPS. Recent data have shown that some members of this family are resident and abundant in the gut of healthy subjects, suggesting that the role of this family should be re-evaluated [26]. Additional work is needed to better understand the signirficance of this group of bacteria in the context of cirrhosis.

Another relevant finding of the present study was the reduced abundance, after TIPS, of bacteria which are usually increased in patients with cirrhosis. In particular, we described a reduction of Clostridiaceae and Actinomycetales, which are responsible of abdominal infections such as hepatic actinomycosis [25,27–29]. Furthermore, at the class level, we found a drop of the levels of Bacilli. In contrast, levels of Bacteroidetes, higher in healthy subjects than in patients with chronic liver diseases [30,31], were increased following the derivative procedure. Taken together, these data indicate that following TIPS, gut microbiota presents a composition more reminiscent of the one present in healthy subjects, supporting the hypothesis that amelioration of portal hypertension has beneficial roles on gut ecology.

A novel line of information provided by the present study is related to the identification of changes in fecal metabolome following TIPS. We analyzed the levels of SCFAs, which are increased in patients with NAFLD, NASH and liver fibrosis [32,33], but TIPS did not induce any significant changes in their levels. This result may be explained considering that after TIPS, patients mainly showed an increase in *Bacteroidaceae* and *Enterobacteriaceae*, which are not relevant SCFAs producing-bacteria [34]. More important, significantly lower levels of MCFAs, specifically isohexanoic, 2-ethylhexanoic and octanoic acids, were measured after TIPS. These results are relevant considering that MCFAs enhance inflammation favoring the secretion of pro-inflammatory cytokines and reducing that of anti-inflammatory factors. Specifically, Sam et al. [5] demonstrated that IL-10 levels decreased in a dose-dependent manner with decanoic acid, which also induced HIF-1α downregulation and increased HIF-2α transcription [5]. In addition, MCFAs can modulate the inflammatory balance favoring differentiation towards T helper (Th) 1 and Th17 [35], usually associated with inflammatory disorders. The pro-inflammatory role of MCFAs has been confirmed in a study where a diet rich in caproic acid (C6:0), caprylic acid (C8:0), and capric acid (C10:0) was found to polarize naive T cells toward Th17 and Th1 phenotype [36]. Moreover, MCFAs are able to bind GPR84, which is expressed by various immune cells [37]. Puengel et al. [38] observed that expression of GPR84 is upregulated in mice and patients with NASH, suggesting a role for MCFAs in the recruitment of inflammatory cells. These data indicate that the proinflammatory state which accompanies decompensated cirrhosis could be ameliorated after TIPS through modification of bacterial metabolites.

The major limitation of this study is related to the low number of patients enrolled. However, this was due to a very stringent patient selection aimed at exploring a well-characterized population in the absence of confounding factors. The characteristics of patients enrolled in the study explain the differences in the results when compared to those recently reported in a group of 106 patients receiving TIPS [20]. In this latter study, no changes in microbial species or families were found comparing the overall population receiving TIPS, and the study was mostly focused on patients who developed HE after the procedure. We decided to exclude patients with HE from our study, as well as we did not enroll any patients receiving systemic or non-absorbable antibiotics. This strict selection allowed us to disclose changes that more confidently reflect the effects of TIPS on gut microbiota. Another major difference with the study published by Li, Tang, al. [20] is related to the ethnic population analyzed and, consequently, to the diet consumed by the patients, which make the conclusions of that study difficult to extrapolate to a Western population, on which our data were obtained. Another difference is related to the timing of stool sample collection. We reasoned that a sample obtained shortly before a programmed procedure could be more representative of a 'steady state' without the influence of acute events. After TIPS, a 12-week interval was considered adequate based on the rapid modifications in splanchnic hemodynamic induced by this procedure, while a longer interval would have been more likely to be influenced by new events.

In our study, nearly 50% pf patients had AUD, and it is well known that alcohol use can a priori alter the GM [39]. However,

all patients enrolled in this series had stopped alcohol intake at least 3 months before TIPS placement (range 5 - 23 months), and many alcohol-induced changes in gut microbiome and metabolome are reversible after a relatively short period of abstinence [40–42]. Finally, it is important to underscore the novel line of information provided in our study by the initial analysis of fecal fatty acids, not conducted in any previous studies. A more extensive evaluation, including different metabolites such as mercaptan indoles or glutamine/glutamate in fecal and plasma samples, should be the objective of future investigation.

In conclusion, reduction of portal hypertension in cirrhotic patient through TIPS placement results in significant changes in gut microbiota, consistent with a healthier ecology, and in a modulation of the levels of fecal fatty acids reflecting a lower pro-inflammatory environment. Future studies are warranted to provide more detailed information on the relationship between gut microbiota composition, metabolic function and long-term clinical outcomes after TIPS.

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Conflict of interest

FV and FF have received lecture fees from Gore. FM and FV have received travel grants from AlfaSigma.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2022.11.017.

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