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Alimentary Tract

Potential biomarkers to predict outcome of faecal microbiota transfer for recurrent *Clostridioides difficile* infection

Fedja Farowski[a,b,1], Philipp Solbach[c,d,e,1], Anastasia Tsakmaklis[a,b], Susanne Brodesser[f], M. Rebeca Cruz Aguilar[a], Oliver A. Cornely[a,b,f], Katja Dettmer[g], Paul G. Higgins[h,b], Sebastian Suerbaum[d,e,i,j], Nathalie Jazmati[b,h], Peter J. Oefner[g], Maria J.G.T. Vehreschild[a,b,*], for the German Clinical Microbiome Study Group (GCMSG)

[a] *Department I of Internal Medicine, University Hospital of Cologne, Germany*
[b] *German Centre for Infection Research (DZIF), Partner Site Bonn-Cologne, Germany*
[c] *Hannover Medical School, Department of Gastroenterology, Hepatology and Endocrinology, Hannover, Germany*
[d] *Hannover Medical School, Institute of Medical Microbiology and Hospital Epidemiology, Hannover, Germany*
[e] *German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Germany*
[f] *Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Germany*
[g] *Institute of Functional Genomics, University of Regensburg, Germany*
[h] *Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Germany*
[i] *Chair of Medical Microbiology and Hospital Epidemiology, Max von Pettenkofer Institute, Faculty of Medicine, LMU Munich, Germany*
[j] *German Center for Infection Research (DZIF), Partner Site Munich, Germany*

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ABSTRACT

Background & Aims: Faecal microbiota transplantation (FMT) has proven high clinical efficacy in the management of recurrent *Clostridioides difficile* infection (rCDI) with cure rates of over 80% after a single treatment. Nevertheless, the reasons for failure in the remaining 20% remain elusive. The aim of the present study was to investigate different potential predictors of response to FMT.
Methods: Faecal specimens of sixteen patients undergoing FMT for rCDI, as well as samples from the respective donors were collected and analyzed by 16S rRNA gene profiling, bile acid-inducible (*baiCD*) gene specific qPCR, and liquid chromatography tandem-mass spectrometry (LC–MS/MS) to quantify the concentrations of primary and secondary bile acids.
Results: Using the faecal concentration of the secondary bile acid lithocholic acid (LCA)within the patient specimens, we were able to predict response to FMT (accuracy 95.2%, sensitivity 100%, specificity 90.9%). By combining the faecal LCA concentration with the urinary pCS concentration, an accuracy of 100% was achieved.
Conclusion: LCA appears to be a promising marker candidate for prediction of clinical response to FMT. Other makers, such as urinary concentration of pCS, but not 3-IS, might be used to improve accuracy of prediction. Further studies are warranted to validate these candidate markers.

1. Introduction

Clostridioides difficile (*C. difficile*) is the main cause of hospital-acquired infectious diarrhea worldwide [1–3]. Treatment of a first *C. difficile* infection (CDI) with oral metronidazole or vancomycin is associated with recurrence rates of 10–25% [4–8]. Current evidence suggests that disruption of the gut microbiota plays a key role in promoting recurrence. While primary bile acids within the gut lumen facilitate *C. difficile* germination, secondary bile acids inhibit vegetative growth and toxin production [9,10]. Transformation of primary into secondary bile acids is mediated by a subpopulation of the gut microbiota [11]. This pathophysiological model explains the high clinical efficacy of faecal microbiota transplantation (FMT) in the management of recurrent CDI (rCDI) with cure rates of over 80% after a single, and over 90% after multiple treatments [12]. While these rates are considerably high, the reasons for failure remain elusive. Assessment of potential biomarkers related to

* Corresponding author at: University Hospital of Cologne, Department I of Internal Medicine, 50924 Cologne, Germany.
E-mail addresses: maria.vehreschild@uk-koeln.de, angelina.lagodny@uk-koeln.de (M.J.G.T. Vehreschild).
[1] These authors contributed equally to this work.

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Table 1
Biomarker results after FMT and in samples of the respective donors.

	Before FMT	Before recurrence	After successful FMT	Donor
Alpha diversity	n = 19	n = 3	n = 35	n = 20
Observed OTUs				
Mean ± SD	29.16 ± 12.05	34.33 ± 10.69	74.63 ± 27.84	70.95 ± 21.33
95% CI	23.35–34.96	7.77–60.90	65.07–84.19	60.97–80.93
Chao1 Index				
Mean ± SD	30.90 ± 13.64	38.00 ± 15.10	79.63 ± 32.20	73.98 ± 23.16
95% CI	24.32–37.47	0.49–75.51	68.57–90.69	63.14–84.82
Inverse Simpson				
Mean ± SD	6.48 ± 4.41	5.82 ± 5.66	13.32 ± 8.13	19.91 ± 8.49
95% CI	4.35–8.60	−8.24–19.89	10.53–16.11	15.94–23.88
Shannon Index				
Mean ± SD	2.22 ± 0.58	2.06 ± 0.84	3.15 ± 0.68	3.37 ± 0.70
95% CI	1.94–2.49	−0.03–4.14	2.92–3.39	3.05–3.70
BaiCD gene	n = 13	n = 2	n = 26	n = 12
positive: n [%]	4 (30.8)	1 (50.0)	20 (76.9)	12 (100)
Bile acids	n = 8	n = 3	n = 10	n = 8
CA [μmol/g faeces]				
Mean ± SD	2.31 ± 1.88	4.96 ± 6.07	0.02 ± 0.03	0.46 ± 1.20
95% CI	0.74–3.87	−10.13–20.05	0.00–0.04	−0.55–1.46
CDCA [μmol/g faeces]				
Mean ± SD	0.77 ± 0.73	1.64 ± 0.95	0.02 ± 0.02	0.16 ± 0.41
95% CI	0.16–1.38	−0.73–4.01	0.00k0.03	−0.18–0.50
DCA [μmol/g faeces]				
Mean ± SD	0.09 ± 0.19	0.12 ± 0.19	1.67 ± 1.44	3.41 ± 3.82
95% CI	−0.06–0.25	−0.35–0.59	0.64–2.69	0.22–6.60
LCA [μmol/g faeces]				
Mean ± SD	0.07 ± 0.19	0.01 ± 0.01	1.60 ± 1.08	1.48 ± 1.41
95% CI	−0.09–0.23	−0.01–0.02	0.83–2.37	0.30–2.66
Relative abundances	n = 19	n = 3	n = 35	n = 20
Clostridiales [%]				
Median [range]	1.6 [0.0–69.5]	6.0 [0.4–10.9]	46.6 [0.5–68.9]	73.2 [7.1–87.1]
Mean ± SD	6.9 ± 15.9	5.7 ± 5.2	41.2 ± 18.8	67.5 ± 18.8
95% CI	−0.7–14.6	−7.3–18.7	34.7–47.7	34.7–47.7
Lachnospiraceae [%]				
Median [range]	0.2 [0.0–33.0]	4.3 [0.0–9.2]	24.0 [0.0–42.6]	34.2 [1.8–65.2]
Mean ± SD	3.6 ± 8.4	4.5 ± 4.6	22.4 ± 11.0	32.8 ± 15.1
95% CI	−0.4–7.8	−6.9–15.9	18.6–26.2	25.8–39.9
Ruminococcaceae [%]				
Median [range]	0.0 [0.0−32.4]	0.1 [0.0−0.4]	13.8 [0.0−34.8]	28.2 [4.2-52.9]
Mean ± SD	1.7 ± 7.4	0.2 ± 0.2	15.0 ± 9.4	28.1 ± 9.9
95% CI	−1.9–5.3	−0.4–0.7	11.7–18.2	23.5–32.7
Urinary metabolites	n = 16	n = 1	n = 19	n = 0
3-IS [μmol/mmol creatinine]				
Mean ± SD	71.22 ± 39.21	19.36	67.84 ± 34.84	
95% CI	50.33–92.12		51.05–84.64	
pCS [μmol/mmol creatinine]				
Mean ± SD	12.13 ± 35.42	1.12	131.97 ± 152.08	
95% CI	−6.75–31.00		58.67–205.27	

3-IS: Indoxyl sulfate; BaiCD: bile acid-inducible CD; CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; pCS: p-cresyl sulfate.

the above described mechanisms may enable early identification of these patients and highlight potential treatment optimization strategies.

Following this line of thought, one could hypothesize that FMT failures present with an inverse primary and secondary bile acid profile, compared to patients with a response to treatment. Evidence in favour of this assumption has been reported from patients undergoing successful FMT for rCDI [13], but there is no data on patients experiencing treatment failure.

Bacteria that facilitate transformation of primary into secondary bile acids may also serve as an indirect marker of FMT success. Key enzymes within the bile acid biosynthesis cascade are encoded by genes within the bile acid-inducible (*bai*) operon [14,15]. 7α-dehydroxylating bacteria, a major source for secondary bile acids in the gut [16], were decreased in rodents treated with ß-lactam antibiotics or quinolones and consecutively lower secondary and higher primary bile acid concentrations were reported [17–20]. We hypothesized that recovery from rCDI might be associated with a return of 7α-dehydroxylating bacteria.

Finally, assessment of metabolites associated with the presence of bacteria considered essential for downregulation of CDI, may help to predict response to FMT. Namely, reduced synthesis of 3-indoxyl sulfate (3-IS) and p-cresyl sulfate (pCS), products of the main pathway of amino acid fermentation in the human large intestine and which are excreted into the urine, is associated with a loss of bacteria from the taxonomic class of Clostridia [21].

Based on these hypotheses, the aim of the present study was to investigate different potential predictors of response to FMT, namely faecal abundance of Clostridia, faecal primary and secondary bile acid concentrations, faecal *baiCD* gene quantification, as well as urinary 3-IS and pCS concentrations.

2. Patients and methods

We analyzed faecal specimens of sixteen patients with rCDI before and after 21 courses of frozen capsulized FMT (one course: 12, two courses: 3, three courses: 1) to identify possible biomarkers predicting treatment outcome. Faecal and urinary specimens were

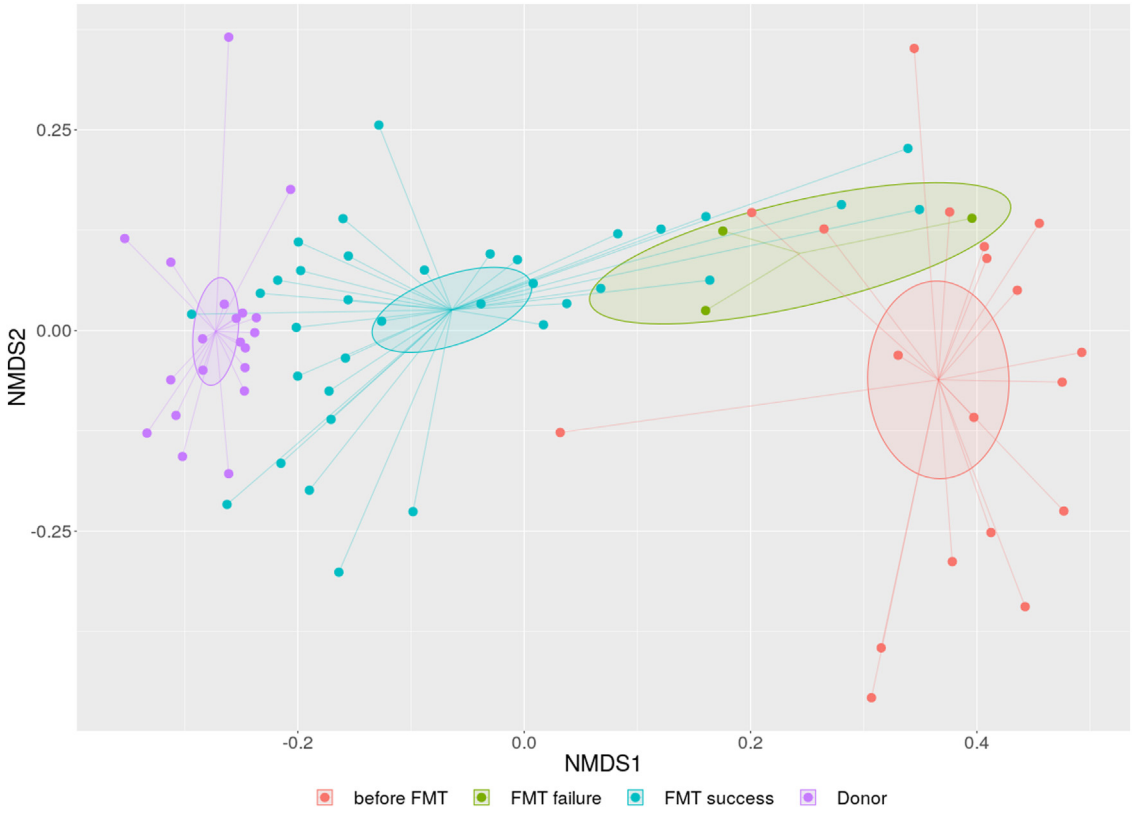

Fig. 1. Non-metric multidimensional scaling (NMDS) plot of Bray–Curtis dissimilarity on abundance data from pre-FMT, post-FMT (failure = recurrence before day 90/success = no recurrence before day 90) and donor faecal samples. Solid ellipses indicate 95% confidence intervals, assuming multivariate t-distribution, around centroids of the respective groups.
FMT = faecal microbiota transfer.

collected at days — 1, 7 and 30 after each course of FMT. Donor specimens were collected at the time of donation. Donor selection and frozen capsulized FMT was performed as published [22]. Patients and donors had signed an informed consent for biobanking and analyses as approved by the local ethics committee (University of Cologne Ethics Committee, 08-160).

DNA was extracted using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH, USA), and the V3-V4 region of the bacterial 16S rRNA gene analysed [23]. The 16S amplicon was purified using the Agencourt AMPure XP PCR Purification system (Beckman Coulter, Krefeld, Germany), processed (indexed, purified, normalized and pooled) and sequenced in a 300-bp paired-end run on the Illumina MiSeq [24].

Sequencing data was processed using the DADA2 pipeline and QIIME version 2 [25,26]. Quality profiles of the reads were analyzed. Reads were trimmed (trunc_len_f = 280, trunc_len_r = 240) and processed by the QIIME DADA2 plugin with the *denoise-paired* option and standard parameters (trunc_q = 2, max_ee = 2, chimera_method = consensus). Rarefaction curves were determined based on the feature table and analysis of the relative proportion of each bacterial taxon was made after the data were rarefied at a depth of 2000 sequences per sample. Taxonomic classification was done by a Naïve Bayes classifier (sklearn) [27], trained on SILVA database release 128 [28].

BaiCD gene measurement was performed with a CFX 96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) and Bio-Rad CFX Manager 3.1 Software. Gene-specific *baiCD* primers (confirmed by testing against DNA of no-target test bacteria) were used [29]. Samples were classified as *baiCD* gene positive by endpoint measurement, when the fluorescent signal was significantly above the background fluorescence.

Quantification of primary and secondary bile acids in pre-FMT and post-FMT (day 7) faecal specimens was performed by liquid chromatography tandem mass spectrometry (LC–MS/MS) using the Biocrates® Life Sciences Bile Acids Kit (Biocartes, Innsbruck, Austria), which covers 16 individual human bile acids [30].

3-IS and pCS concentrations in urinary specimens were determined by LC–MS/MS and adapted to kidney function [21].

Statistical analyses were carried out using R for Statistical Computing (version 3.2.5, R Foundation for Statistical Computing, Vienna, Austria) [31]. The QIIME biom data was imported and diversity scores calculated using the phyloseq R package [32]. All continuous data was presented as mean and standard deviation (SD) or median and range, presented as box plots and tested with Mann–Whitney U-test and Kruskal–Wallis-test with Dunn's post test, as appropriate. Receiver operating characteristic (ROC) analysis was performed using the ROCR package [33]. All statistical tests were two-tailed, and a P value of <0.05 was considered statistically significant.

3. Results

Overall, 16 patients (age 67.6 ± 13.9 years, 10/16 (62.5%) female) underwent 21 courses of FMT for rCDI. Three patients (18.8%) experienced another recurrence at day 8 and 42, respectively. Thirteen of 16 patients (81%) experienced no recurrence within 90 days after a single FMT. Two patients with a recurrence before day 90 received an additional FMT. Two patients experienced a recurrence after day 90. Summarized and detailed patient characteristics are shown in supplementary Tables S1 and S2, respectively. Biomarker results at different time points are shown in Tables 1 and S3. Alpha diversity in faecal specimens was significantly increased following success-

Table 2
Biomarker results by bile acid-inducible (*baiCD*) gene status.

	BaiCD negative	*BaiCD* positive	Mann–Whitney U-test
Alpha diversity	n = 16	n = 25	
Observed OTUs			
Mean ± SD	35.13 ± 20.53	73.28 ± 33.16	p < 0.001
95% CI	24.19–46.06	59.59–86.97	
Chao1 Index			
Mean ± SD	35.65 ± 20.88	78.42 ± 37.53	p < 0.001
95% CI	24.52–46.78	62.93–93.92	
Inverse Simpson			
Mean ± SD	5.87 ± 3.90	14.18 ± 8.80	p < 0.001
95% CI	3.79–7.95	10.55–17.81	
Shannon Index			
Mean ± SD	2.24 ± 0.63	3.10 ± 0.86	p < 0.001
95% CI	1.91–2.58	2.75–3.46	
Bile acids	n = 6	n = 12	
CA [μmol/g faeces]			
Mean ± SD	4.13 ± 4.31	0.47 ± 1.04	p < 0.01
95% CI	−0.39–8.66	−0.19–1.13	
CDCA [μmol/g faeces]			
Mean ± SD	1.23 ± 1.02	0.21 ± 0.46	p < 0.01
95% CI	0.16–2.31	−0.08–0.51	
DCA [μmol/g faeces]			
Mean ± SD	0.60 ± 1.45	1.14 ± 1.29	p = 0.055
95% CI	−0.93–2.12	0.33–1.96	
LCA [μmol/g faeces]			
Mean ± SD	0.30 ± 0.71	1.22 ± 1.18	p = 0.125
95% CI	−0.45–1.05	0.46–1.97	
Relative abundances	n = 16	n = 25	
Clostridiales [%]			
Median [range]	1.3 [0.2–58.0]	46.6 [0.0–68.9]	
Mean ± SD	9.3 ± 16.2	38.4 ± 22.5	p < 0.001
95% CI	0.6–17.9	29.2–47.7	
Lachnospiraceae [%]			
Median [range]	0.1 [0.0–41.4]	23.0 [0.0–42.6]	
Mean ± SD	5.5 ± 11.2	20.4 ± 12.3	p < 0.001
95% CI	−0.5–11.4	15.3–25.5	
Ruminococcaceae [%]			
Median [range]	0.0 [0.0–14.8]	14.8 [0.0–34.8]	
Mean ± SD	2.3 ± 4.9	14.9 ± 11.0	p < 0.001
95% CI	−0.3–4.9	10.4–19.4	
Urinary metabolites	n = 13	n = 19	
3-IS [μmol/mmol creatinine]			
Mean ± SD	83.23 ± 42.15	59.72 ± 32.06	p = 0.071
95% CI	57.76–108.70	44.27–75.17	
pCS [μmol/mmol creatinine]			
Mean ± SD	66.98 ± 141.96	90.13 ± 129.90	p = 0.084
95% CI	−18.80–152.76	27.52–152.70	

3-IS: Indoxyl sulfate; BaiCD: bile acid-inducible CD; CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; pCS: p-cresyl sulfate.

ful FMT ($p < 0.01$, Table 1 and Figs. S1A and S2) compared patients experiencing recurrence.

In terms of beta diversity, unweighted, weighted and generalized UniFrac distances, as well as Bray–Curtis Non-metric MultiDimensional Scaling (NMDS) (Fig. 1 and Fig. S3) showed a marked shift of the recipient gut microbiota composition towards the donor gut microbiota composition after successful FMT ($p < 0.001$). However, based on the NMDS-plots it was not possible to clearly assign all samples to either of the two groups. The shift in microbiota composition after FMT is characterized by an increase of bacteria from the order of Clostridiales ($p < 0.001$), with Lachnospiraceae ($p < 0.001$) and Ruminococcaceae ($p < 0.001$) being the prevalent families. The abundance of bacteria from these families did not increase in samples from patients who experienced a subsequent recurrence of CDI (Table 1 and Fig. S4).

For an indirect detection of bacterial species converting primary into secondary bile acids, we performed PCR to detect the presence of the *baiCD* gene in the faecal specimens. All tested donor samples were *baiCD* gene positive (Table 1). The proportion of *baiCD* gene positive samples increased significantly after successful FMT ($p < 0.05$) when compared with samples obtained before FMT. However, one of two post FMT samples of patients who experienced a recurrence before day 90 was *baiCD* gene positive. The alpha diversity, as well as the relative abundance of bacteria from the order of Clostridiales, were significantly increased in *baiCD* gene positive samples (Table 2; $p < 0.001$ for both).

Bile acid analysis on day 7 revealed a significant decrease in the concentration of the primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) after successful FMT ($p < 0.01$ for both; Fig. 2). Correspondingly, the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA) increased significantly ($p < 0.01$ for both; Fig. 2). Faecal primary and secondary bile acid concentrations before FMT and before recurrence of CDI were comparable; as well as in faecal samples after successful FMT and donor samples.

baiCD gene positive samples exhibited significantly lower concentrations of the primary bile acids CA ($p < 0.01$) and CDCA ($p < 0.01$). Urinary pCS concentrations were significantly increased following successful FMT ($p < 0.001$; Fig. S1B), but not urinary 3-IS concentrations ($p > 0.05$; Fig. S1C).

To assess sensitivity and specificity for the prediction of response to FMT by use of the assessed biomarkers on day 7, we

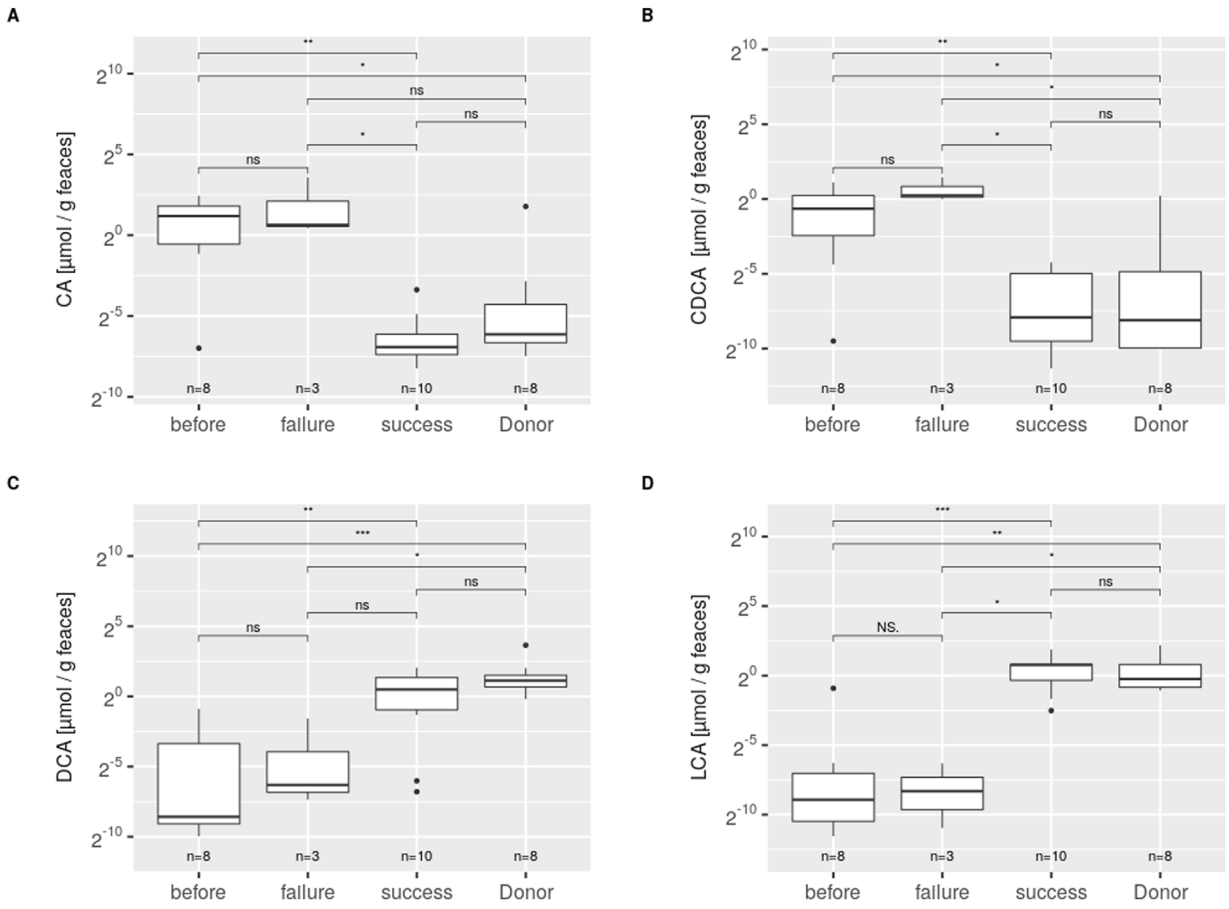

Fig. 2. Faecal concentration of primary (A, cholic acid (CA); B, chenodeoxycholic acid (CDCA)) and secondary (C, deoxycholic acid (DCA); D, lithocholic acid (LCA)) bile acids in pre-FMT, post-FMT (failure = recurrence before day 90/success = no recurrence before day 90) and donor samples, compared using the Mann–Whitney U test. *, $p < 0.05$; **, $p < 0.01$; ns, not significant ($p > 0.05$).
FMT = faecal microbiota transfer.

performed ROC analyses (Fig. 3). Since the incidence and hence the amount of failures was low, we choose to classify the samples as successful FMT (post-FMT without recurrence) versus not successful FMT (pre-FMT + post-FMT with recurrence). The area under the curve (AUC) of LCA was 98% followed by pCS (92%), DCA (91%), the relative abundance of Clostridia (90%) and 3-IS (47%). Presence of the *baiCD* gene predicted a successful FMT with a negative and positive predictive value of 60% (NPV) and 83% (PPV), respectively and an accuracy (ACC) of 74%. By combining the urinary concentration of pCS and the faecal concentrations of LCA and DCA, the AUCs were increased to 100% and 97%, respectively. Hence, the faecal concentration of LCA, as well as the combination of LCA with pCS, were the most promising candidates for a biomarker predicting response to FMT. The respective waterfall plots are based on the following categories: pre-FMT plus post-FMT with recurrence versus post-FMT with treatment success (Fig. 4 and Fig. S5). A value greater than −4.0 for the binary logarithm of the faecal LCA [µmol] per g faeces (i.e. greater than 0.0625 µmol/g faeces) predicts response to FMT (NPV: 91%, PPV: 100%, ACC: 95%). The optimal cut off for the combination of LCA with pCS ($\log_2$ (LCA [µmol/g feaces]) + $\log_2$ (pCS [µmol/mmol creatinine)) was 3.75 (NPV: 100%, PPV: 100%, ACC: 100%). The accuracies for different cut off values are shown in the supplementary Fig. S6.

4. Discussion

FMT is a highly effective treatment option for patients suffering from rCDI. However, up to 20% of patients undergoing a single FMT experience a subsequent recurrence [34]. Recently published data suggests that inpatient status, presence of severe or severe-complicated CDI, cirrhosis, as well as previous CDI-related hospitalizations, are associated with FMT failure [35,36]. However, these clinical factors cannot be influenced and therefore are unsuitable for therapeutic manipulation.

In contrast, our assessment of a comprehensive set of potential biomarkers shows that prediction of response to FMT may be feasible. The combination of the urinary concentration of pCS and the faecal concentrations of LCA, showed the highest predictive value for FMT success. Consequently, these biomarkers may be used to identify FMT failure at an early stage. Our findings are limited by the restricted volume of stool available from each patient, which led to low patient numbers for some constellations, especially for those patients without response to treatment.

Overall, our findings are fully in line with the rapidly evolving basis of evidence on the regulation of CDI. As previously shown, alpha diversity in faecal specimens increased significantly following a successful, but not an unsuccessful FMT [37–40].

In 2015, Buffie et al. showed that *Clostridium scindens* mediates CDI resistance in a mouse model, and confirmed this observation in haematological patients [41]. The suggested underlying mechanism includes the possible involvement of *C. scindens* in secondary bile acid biosynthesis and its role in overcoming colonization resistance against *C. difficile*. One of the key enzymes in converting primary to secondary bile acids is an NADH:flavin-dependent oxidoreductase, encoded by the *baiCD* gene within the bile-acid inducible (*bai*) operon [14]. We hypothesized that *baiCD*

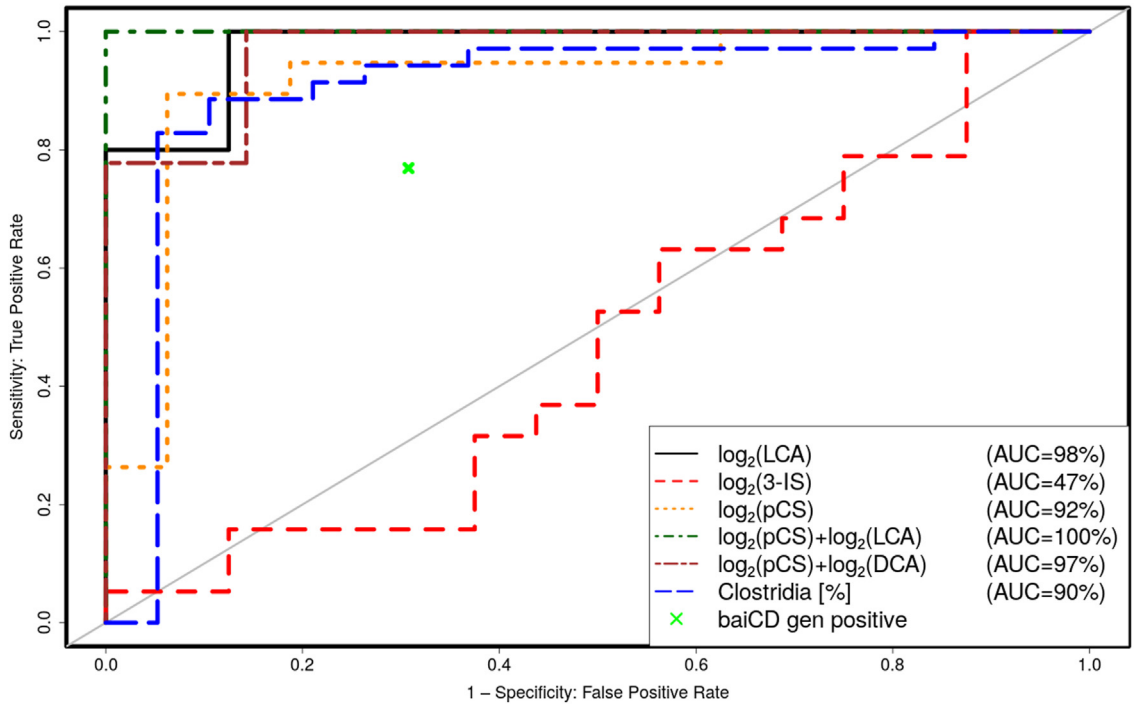

Fig. 3. Receiver operating characteristic curves (ROC) differentiating faecal specimens collected after pre-FMT + post-FMT with recurrence vs. post-FMT with treatment success. Binary logarithms of faecal lithocholic acid [$\log_2$(LCA)], urinary 3-indoxyl sulfate concentration [$\log_2$(3-IS)], urinary p-cresyl sulfate concentration [$\log_2$(pCS)], relative abundance of bacteria from the class of Clostridia, and the sum of the binary logarithms of pCS and LCA [$\log_2$(pCS) + $\log_2$(LCA)] are shown. X indicates the respective values for *baiCD* positivity.
FMT = faecal microbiota transfer.

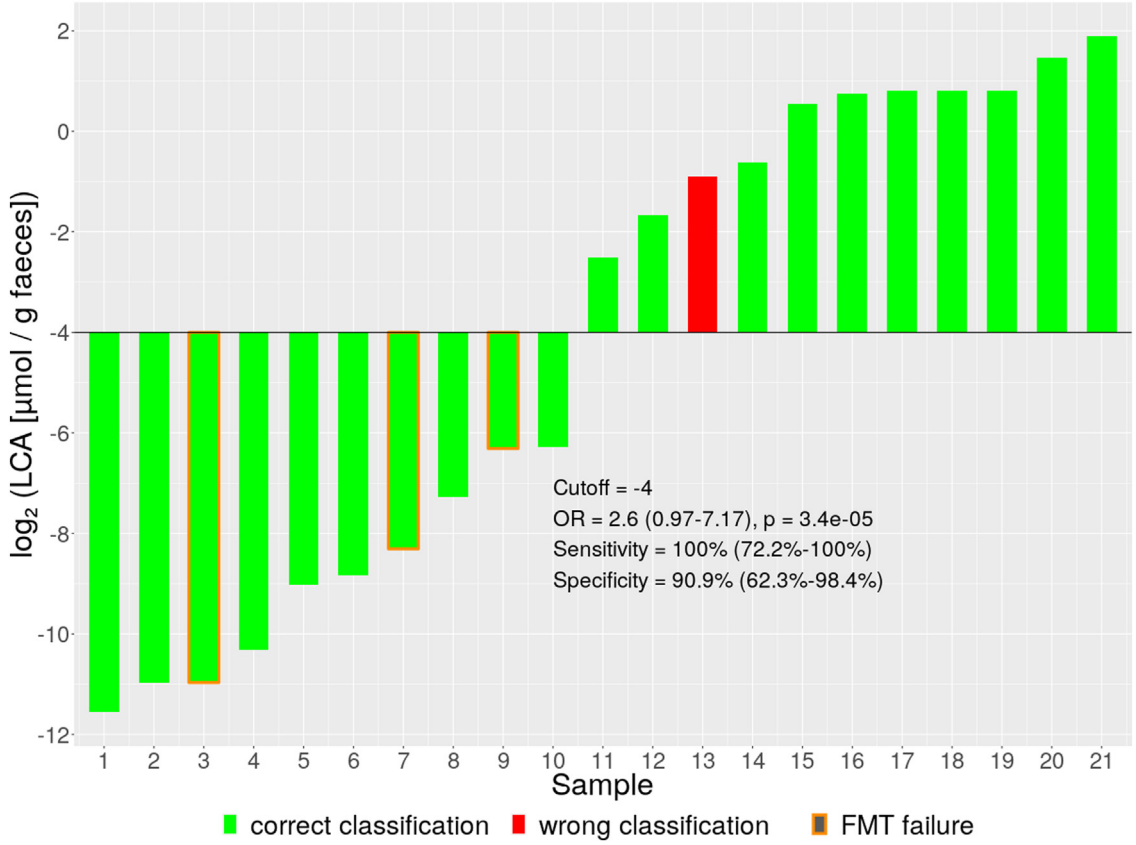

Fig. 4. Classification of outcome (pre-FMT + post-FMT with recurrence vs. post-FMT with treatment success) based on the faecal concentration of lithocholic acid (LCA). Colour code: green: correct, red: wrong, orange border: FMT failure.
FMT = faecal microbiota transfer.

gene positive species could play a role in FMT success or failure. We showed that all faecal donor samples tested positive for the *baiCD* gene (100%) in contrast to the recipients' faecal samples (30.8%). This value increased to 76.9% after successful FMT. However, 4/13 patients suffering from rCDI were positive for the *baiCD* gene (*C. scindens* and *Clostridium hiranonis*). Hence, the stand-alone protective impact of these species in mediating *C. difficile* activity was not confirmed. Recently, Solbach et al. showed that faecal samples of CDI patients were less frequently *baiCD* positive compared to *C. difficile* negative faecal samples (72.5% vs. 35.9%) [29]. However, the negative predictive value in this study was low and faecal samples of patients who experienced a subsequent recurrence after FMT were *baiCD* gene positive, which argues against protective effects.

In addition, we analyzed the faecal bile acid profile before and 7 days after FMT and showed that the primary bile acid CA was significantly decreased, while the secondary bile acid LCA was significantly increased in patients with response to treatment. Our data are in accordance with Weingarden et al. who found primary bile acids CA and CDCA to be present in significant amounts in pre-FMT samples only, while the secondary bile acids DCA and LCA were absent in those samples and only detected in post-FMT samples of 12 CDI patients and in samples from the respective donors. Similar results were obtained in another study, where primary and secondary bile acid concentrations of patients with rCDI and from a first episode of CDI were compared with those of healthy individuals [42].

The intestinal microbiota influences individual serum levels of indoxyl sulfate and p-cresyl sulfate. Weber et al. showed that high urinary 3-IS levels are associated with bacteria from the families of Lachnospiraceae and Ruminococcaceae, which belong to the order of Clostridiales [21]. In our study, the levels of p-cresyl sulfate were significantly lower before FMT and significantly increased post FMT ($p < 0.001$). Interestingly, the patient who experienced a recurrence and had a urine sample available, showed no increase in of the urinary p-cresyl sulfate concentration. Hence, the levels of p-cresyl sulfate in the urine maybe indicate a recovery process to a more balanced microbiota composition after FMT.

After analyzing these metabolic profiles, the combination of different metabolic markers, i.e. urinary concentration of pCS and faecal concentration of LCA, predicted success of FMT in 100% of cases. This result may reflect that response to FMT is a multifactorial process.

Even though our results offer a promising perspective, validation of a larger sample set including sufficient cases of FMT failures is warranted. Such a study should also integrate associated clinical risk factors into the prediction model. Another issue requiring further exploration are the clinical consequences of predicting FMT failure. A second pre-emptive FMT based on preparations from a different donor could be considered, but does not represent a realistic option within the current regulatory circumstances of FMT application. Ideally, however, understanding the predictors of FMT success and failure may facilitate the development of FMT modifications that will be able to prevent the latter.

If the markers described in this study were successfully validated in the future, one might also consider assessing their potential to predict primary CDI in patients scheduled for antibiotic exposure.

Conflict of interest

MRCA received travel funding from Gilead.

OAC has received research grants from Actelion, Amplyx, Arsanis, Astellas, AstraZeneca, Basilea, Bayer, Cidara, F2G, Gilead, GSK, Leeds University, Matinas, Medicines Company, MedPace, Melinta, Merck/MSD, Miltenyi, Pfizer, Rempex, Roche, Sanofi Pasteur, Scynexis, Seres, is a consultant to Allecra Therapeutics, Amplyx, Actelion, Astellas, Basilea, Cidara, Da Volterra, F2G, Gilead, IQVIA, Janssen, Matinas, Menarini, Merck/MSD, Paratek, PSI, Scynexis, Seres, Summit, Tetraphase, Vical, and received lecture honoraria from Astellas, Basilea, Gilead, Merck/MSD and Pfizer.

NJ has received payment for lectures from MSD Sharp & Dohme and travel support from IMDx (Qiagen).

MJGTV is a consultant to: Alb-Fils Kliniken GmbH, Arderypharm, Astellas Pharma, Berlin Chemie, DaVolterra, MaaT Pharma and Merck/MSD; has served at the speakers' bureau of: Astellas Pharma, Basilea, Gilead Sciences, Merck/MSD, Organobalance, Pfizer; received research funding from: 3M, Astellas Pharma, DaVolterra, Gilead Sciences, MaaT Pharma, Merck/MSD, Morphochem, Organobalance, Seres Therapeutics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2019.01.012.

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