CME

Predictors of Early Failure After Fecal Microbiota Transplantation for the Therapy of *Clostridium Difficile* Infection: A Multicenter Study

Monika Fischer, MD, MSc[1], Dina Kao, MD[2], Shama R. Mehta, MD[2], Tracey Martin, MD[3], Joseph Dimitry[2], Ammar H. Keshteli[2], Gwendolyn K. Cook, MS[1], Emmalee Phelps[1], Brian W. Sipe, MD[1], Huiping Xu, PhD[4] and Colleen R. Kelly, MD[3]

OBJECTIVES: **Fecal microbiota transplant (FMT) is a highly efficacious treatment for recurrent or refractory *Clostridium difficile* infection (CDI); however, 10–20% of patients fail to achieve cure after a single FMT. The aim of this study was to identify risk factors associated with FMT failure and to develop and validate a prediction model for FMT failure.**

METHODS: **Patient characteristics, CDI history, FMT characteristics, and outcomes data for patients treated between 2011 and 2015 at three academic tertiary referral centers were prospectively collected. Early FMT failure was defined as non-response or recurrence of diarrhea associated with positive stool *C. difficile* toxin or PCR within 1 month of FMT. Late FMT failure was defined as recurrence of diarrhea associated with positive stool *C. difficile* toxin or PCR between 1 and 3 months of the FMT. Patient data from two centers were used to determine independent predictors of FMT failure and to build a prediction model. A risk index was constructed based on coefficients of final predictors. The patient cohort from the third center was used to validate the prediction model.**

RESULTS: **Of 328 patients in the developmental cohort, 73.5% (*N*=241) were females with a mean age of 61.4±19.3 years; 19.2% (*N*=63) had inflammatory bowel disease (IBD), and 23.5% (*N*=77) were immunocompromised. The indication for FMT was recurrent CDI in 87.2% (*N*=286) and severe or severe-complicated in 12.8% (*N*=42). FMT was performed as an inpatient in 16.7% (*N*=54). The stool source was patient-directed donors in 40% (*N*=130) of cases. The early FMT failure rate was 18.6%, and the late failure rate was 2.7%. In the multivariable analysis, predictors of early FMT failure included severe or severe-complicated CDI (odds ratio (OR) 5.95, 95% confidence interval (CI): 2.26–15.62), inpatient status during FMT (OR 3.78, 95% CI: 1.55–9.24), and previous CDI-related hospitalization (OR 1.43, 95% CI: 1.18–1.75); with each additional hospitalization, the odds of failure increased by 43%. Risk scores ranged from 0 to 13, with 0 indicating low risk, 1–2 indicating moderate risk, and ≥3 indicating high risk. In the developmental cohort, early FMT failure rates were 5.6% for low risk, 12.7% for moderate risk, and 41% for high-risk patients. Of 134 patients in the validation cohort, 57% (*N*=77) were females with a mean age of 66±18.1 years; 9.7% (*N*=13) had IBD, and 17.9% (*N*=24) were immunocompromised. The early FMT failure rate at 1 month was 19.4%, with an additional 3% failing by 3 months. In the validation cohort, FMT failure rates were 2.1% for low risk, 16.1% for moderate risk, and 35.7% for high risk patients. The area under the receiver operating characteristic curve (AUROC) for FMT failure was 0.81 in the developmental cohort and 0.84 in the validation cohort.**

[1]Division of Gastroenterology and Hepatology, Indiana University, Indianapolis, Indiana, USA; [2]Department of Gastroenterology, Zeidler Ledcor Centre, University of Alberta, Edmonton, Alberta, Canada; [3]Lifespan Women's Medicine Collaborative, Division of Gastroenterology, Alpert Medical School of Brown University, Providence, Rhode Island, USA; [4]Department of Biostatistics, The Richard M. Fairbanks School of Public Health and School of Medicine, Indiana University, Indianapolis, Indiana, USA. **Correspondence:** Monika Fischer, MD, MSc, Division of Gastroenterology and Hepatology, Indiana University, 550 North University Boulevard, Suite 1602, Indianapolis, Indiana 46202, USA. E-mail: mofische@iu.edu
Received 8 December 2015; accepted 11 April 2016

CONCLUSIONS: **Severe and severe-complicated indication, inpatient status during FMT, and the number of previous CDI-related hospitalizations are strongly associated with early failure of a single FMT for CDI. The novel prediction model has good discriminative power at identifying individuals who are at high risk of failure after FMT therapy and may assist the treating physician in subsequent management plans.**

Am J Gastroenterol 2016; 111:1024–1031; doi:10.1038/ajg.2016.180; published online 17 May 2016

INTRODUCTION

Clostridium difficile infection (CDI) is one of the most common hospital-acquired infections in developed countries, with increasing incidence, severity, and mortality over the last decade. Following a course of standard antibiotic therapy for CDI, approximately 20–30% of patients will experience a recurrence. The risk of recurrence continues to rise with each subsequent episode, approaching 50–60% after the third episode (1). Managing patients with recurrent CDI is a major clinical challenge, as standard antibiotic therapy often proves largely ineffective.

Fecal microbiota transplantation (FMT), which aims to restore intestinal microbiota and colonization resistance, has emerged as the most effective alternative in the management of recurrent CDI, although the precise mechanism of cure remains unknown (2–4). Three randomized controlled trials and four systematic reviews demonstrated a cure rate of at least 80% (5–10). The efficacy appears to be higher when FMT is infused by the lower route and with larger stool inoculums compared with the upper route of delivery or with a lower volume of fecal slurry (11). Fresh or frozen and thawed FMT (12,13) and volunteer or universal stool donors (11) appear to offer similar efficacy rates, and stool banks have been developed to provide material for FMT more efficiently.

Although highly efficacious for recurrent CDI, approximately 10–20% of patients will fail the first FMT, and a smaller proportion may not respond to FMT at all despite multiple treatments (11). The cause of FMT failure in the treatment of CDI is not known. A few small studies have suggested potential patient characteristics contributing to FMT failure, such as advanced age (14), severity of CDI (15), and immunocompromised state (16).

In this multicenter study, we aimed to identify risk factors associated with FMT failure. We developed a predictive model and built a risk index for FMT failure based on the model. We then validated the index in an independent cohort of patients. Our goal was to accurately stratify patients into low-, moderate-, and high-risk groups for FMT failure based on patient and procedure characteristics before or at the time of FMT.

METHODS

Study cohort

This retrospective study included adults (≥18 years) who received FMT for recurrent, severe, or severe-complicated CDI between January of 2011 and March of 2015 at University Hospital, Indiana University, Indianapolis, IN, Women's Medicine Collaborative, The Miriam Hospital, Brown University, Providence, RI, and University of Alberta Hospital, Edmonton, AB, Canada. Each center is known for high volume FMT, treating at least 80 cases of CDI per year, and maintains a prospective program database.

Recurrent CDI was defined as at least three episodes of CDI and failure of a 6- to 8-week vancomycin taper or pulse-dosed therapy or at least two episodes of CDI requiring hospitalization. Severe CDI was defined as serum albumin concentration <3 g/dl and the presence of either of the following: abdominal tenderness or white blood cell count (WBC) >15,000 cells/mm^3. Severe-complicated CDI was defined as any of the following: admission to an intensive care unit for CDI, hypotension with or without the required use of vasopressors, fever ≥38.5 °C, ileus or significant abdominal distension, mental status changes, WBC >35,000 or <2000 cells/mm^3, serum lactate >2.2 mmol/l, and end-organ dysfunction (17).

Early FMT failure was defined as non-response or recurrence of diarrhea associated with positive stool *C. difficile* toxin or PCR within 1 month of FMT. Late FMT failure was defined as recurrence of diarrhea associated with positive stool *C. difficile* toxin or PCR between 1 and 3 months post FMT. FMT was delivered predominately by colonoscopy. Both patient-directed and universal stool donors as well as fresh or frozen and thawed stools were used, depending on availability. Sporicidal disinfection was performed in patient rooms as per institutional protocols; specific instructions with handouts regarding sporicidal home cleaning were provided to patients to prevent reinfection. Post-FMT follow-up included either clinic visits or phone calls at intervals that varied by site to assess for short- and long-term success. Patients who did not complete at least 3 months of follow-up post FMT at the time of the data extraction were excluded.

Immunocompromised state was defined as any of the following: HIV infection (any CD4 count), AIDS-defining diagnosis or CD4 <200/mm^3, inherited or primary immune disorders, ongoing treatment with anti-neoplastic agents or immunosuppressant medications (including but not limited to monoclonal antibodies to B and T cells, anti-tumor necrosis factor agents, systemic steroids (≥20 mg prednisone/day), antimetabolites (azathioprine, 6-mercaptopurine, methotrexate), calcineurin inhibitors (tacrolimus, cyclosporine), and mycophenolate mofetil). Data were abstracted from electronic medical records, telephone interviews, and FMT program databases. To develop and test the performance of the prediction model for FMT failure, we separated these individuals into developmental and validation cohorts. The developmental cohort included patients from Indiana and Brown Universities, and the validation cohort included patients from University of Alberta. The Institutional Review Boards of all three participating centers approved the study.

Risk factors for FMT failure

Potential risk factors associated with FMT failure were divided into three categories: patient characteristics, CDI history and

management, and FMT variables. These risk factors were chosen based on clinical relevance and previous reports.

1. Patient characteristics included patient age at the time of FMT, gender, underlying inflammatory bowel disease (IBD), immunocompromised state, the use of antisecretory therapy such as proton-pump inhibitor or H2-blocker, diverticulosis, prior history of colectomy or presence of ileal-pouch anal anastomosis, and the use of non-CDI antibiotic within 8 weeks of FMT.
2. CDI history and management included a number of prior CDI episodes, history and a number of CDI-related hospitalizations, CDI treatment, and the use of CDI antibiotic or probiotic within 8 weeks of FMT, inpatient vs. outpatient status, relevant laboratory data such as WBC and serum albumin concentration, as well as the presence or absence of pseudomembranes at the time of FMT.
3. FMT variables included indication for FMT (recurrent, severe, or severe-complicated CDI), donor type (patient directed vs. volunteer), stool type (fresh or frozen), and method or location of delivery (NG tube, sigmoidoscopy vs. colonoscopy; beyond or below splenic flexure).

Prediction model development, validation, and statistical analysis

The first step in the development of the prediction model involved evaluating the bivariable association between each risk factor and FMT failure. Summary statistics were calculated for patients with FMT failure and those with success. Comparison was performed using the non-parametric Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. A multivariable logistic regression analysis was performed to determine the final predictors for FMT failure. All potential risk factors were included into the model with a forward stepwise variable selection method. The cutoff *P* value of 0.01 was used in the stepwise variable selection procedure to determine when to stop selecting more factors into the model. To examine the appropriate functional form of the effect that continuous variables had on FMT failure, we divided each continuous variable into intervals with equal length. We calculated the failure rate for each interval and plotted the log-odds of FMT failure against the intervals.

Finally, a risk scoring system was constructed to assign points to each risk factor in the final multivariate logistic regression model. We calculated the risk points associated with each risk factor by dividing the logarithm of the corresponding odds ratio by the lowest logarithms of odds ratios in the model and rounding to the nearest integer. We computed the risk score for each patient by adding the points for each risk factor. Risk groups were created based on the distribution of risk points in the developmental cohort and patients were divided into low-, moderate-, and high-risk groups of approximately equal size according to their risk scores.

To validate the prediction model and the risk scoring system created based on developmental cohort, we applied the point scoring system to the validation cohort by determining the risk points for each patient. The performance of the risk scoring system was evaluated by calculating the area under the receiver operating characteristic curve (AUROC) and comparing the failure rates for risk groups in the developmental and validation cohorts. All statistical analyses were performed using SAS version 9.4 (SAS, Cary, NC).

RESULTS

The developmental cohort included a total of 328 patients, with 179 patients from IU and 149 patients from Brown. The early FMT failure rate was 18.6% (*N*=61). An additional 2.7% (*N*=9) of patients had CDI-related symptoms again (late FMT failure) between 1 and 3 months post FMT. The majority of FMT failure cases (87% (61/70)) were in the early failure group. Patients with severe (72%, *N*=19) or severe-complicated (52.9%, *N*=9) CDI were more likely to have early failure than those with recurrent CDI (11.9%, *N*=34). All failures but one in severe and severe-complicated CDI occurred within 1 month and during the hospitalization when FMT was given; some had temporary clinical improvement within 24–48 h, but all needed additional anti-CDI antibiotics ± repeat FMT(s) to achieve complete symptom resolution. As the large majority of failures occurred within 1 month of FMT, further analysis was based on the early failure outcome.

Patient baseline characteristics of the developmental cohort are shown in **Table 1**. The average age was 61.4 years with a female predominance. A total of 87.2% of the patients received FMT for recurrent CDI, whereas 12.8% of patients met the criteria for severe or severe-complicated disease. Of the latter group, 25 patients had severe and 17 had severe-complicated CDI indication. A total of 16% (*N*=54) were inpatients with 85% (*N*=46) receiving fresh stool, of which 83% (*N*=38) was obtained from on-site healthy volunteers. Seventy-seven (23.5%) patients were considered immunocompromised. Of these, 3 patients had common variable immunodeficiency syndrome, 3 selective IgA deficiency, and 71 received immunosuppressive therapy for the following conditions: 20 patients for solid organ transplant, 29 for IBD, 6 for rheumatoid arthritis, 2 for lupus, 1 for bullous pemphigoid, 1 for severe chronic obstructive pulmonary disease, 1 for psoriasis, and 11 patients received chemotherapy for malignancy.

Several variables were found to be associated with early FMT failure in the univariate analysis. These included the use of non-CDI antibiotics within 8 weeks of FMT, a history of CDI-related hospitalization, a number of CDI-related hospitalizations, severe or severe-complicated CDI, pseudomembranous colitis, serum albumin concentration, and inpatient FMT. Patients with probiotic use before FMT, stool delivery beyond the splenic flexure via colonoscopy, and patient-directed stool donor were more likely to have success at 1 month.

On the basis of a multivariable logistic regression model, we identified three final predictors of early FMT failure: severe or severe-complicated CDI, inpatient status at FMT, and a number of prior CDI-related hospitalizations (**Table 2**). Patients with severe or severe-complicated CDI were six times as likely to fail as patients with recurrent disease. Patients with FMT performed in the inpatient setting were nearly four times as likely to fail as those with FMT performed in the outpatient setting. In addition, patients

Table 1. Baseline characteristics of the developmental cohort at the time of FMT

	Total (*N*=328)	Success at 1 month (*N*=267)	Failure at 1 month (*N*=61)	Odds ratio (95% confidence interval)
Patient characteristics				
Age, mean (s.d.)	61.45 (19.30)	60.76 (19.56)	64.51 (17.94)	1.01 (0.99–1.03)
Female gender, *n* (%)	241 (73.5%)	196 (73.4%)	45 (73.8%)	1.02 (0.54–1.92)
IBD, *n* (%)	63 (19.2%)[a]	50 (18.7%)	13 (21.3%)	1.18 (0.59–2.33)
Crohn's disease, *n* (%)	33 (10.1%)	26 (9.7%)	7 (11.5%)	1.2 (0.5–2.19)
Ulcerative colitis, *n* (%)	25 (7.6%)	22 (8.2%)	3 (4.9%)	0.58 (0.17–1.99)
Immunocompromised state, *n* (%)	77 (23.5%)	60 (22.5%)	17 (27.9%)	1.33 (0.71–2.5)
Diverticulosis, *n* (%)	118 (36.0%)	95 (35.6%)	23 (37.7%)	1.1 (0.62–1.95)
Colectomy, *n* (%)	25 (7.6%)	21 (7.9%)	4 (6.6%)	0.82 (0.27–2.49)
Use of non-anti-CDI antibiotics within 8 weeks of FMT, *n* (%)	36 (11.0%)	24 (9.0%)	12 (19.7%)	2.48 (1.16–5.29)
Anti-secretory therapy before FMT, *n* (%)	117 (35.7%)	93 (34.8%)	24 (39.3%)	1.21 (0.69–2.15)
CDI history and management				
Presence of CDI-related hospitalization before FMT, *n* (%)	180 (54.9%)	137 (51.3%)	43 (70.5%)	2.27 (1.24–4.13)
Number of CDI-related hospitalization before FMT, mean (s.d.)	1.16 (1.45)	1.03 (1.38)	1.75 (1.57)	1.36 (1.14–1.62)
Number of CDI episodes before FMT, mean (s.d.)	3.93 (2.23)	3.97 (2.13)	3.75 (2.64)	0.97 (0.84–1.09)
Use of probiotic before FMT, *n* (%)	189 (57.6%)	162 (60.7%)	27 (44.3%)	0.52 (0.29–0.9)
Use of CDI antibiotics within 8 weeks of FMT, *n* (%)	317 (96.6%)	257 (96.3%)	60 (98.4%)	2.33 (0.29–18.59)
Presence of pseudomembranes at FMT, *n* (%)	21 (6.4%)	5 (1.9%)	16 (26.2%)	18.63 (6.5–53.39)
Inpatient FMT, *n* (%)	54 (16.7%)	24 (9.1%)	30 (50.0%)	10 (5.18–19.3)
WBC at FMT, mean (s.d.)	11.37 (10.54)	10.31 (9.72)	14.21 (12.17)	1.03 (1–1.07)
Albumin at FMT, mean (s.d.)	3.44 (0.83)	3.64 (0.77)	2.87 (0.73)	0.27 (0.16–0.48)
FMT				
FMT indication, *n* (%)				
Recurrent	286 (87.2%)	252 (94.4%)	34 (55.7%)	1.00 (Reference)
Severe	25 (7.6%)	7 (2.6%)	18 (29.5%)	13.34 (6.46–49)
Severe/Complicated	17 (5.2%)	8 (3%)	9 (14.8%)	
Stool delivery: beyond splenic flexure, *n* (%)	249 (76.9%)	210 (79.8%)	39 (63.9%)	0.45 (0.25–0.82)
Stool donor type: patient directed, *n* (%)	130 (39.8%)	114 (42.7%)	16 (26.7%)	0.49 (0.26–0.91)

CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation; IBD, inflammatory bowel disease; WBC, white blood cell count.
[a]Among the IBD patients, five were classified as indeterminate colitis.

with a greater number of CDI-related hospitalizations before FMT were more likely to have a failure outcome. The number of CDI-related hospitalizations was found to have a linear relation with the risk of FMT failure, with each additional hospitalization increasing the odds of failure by 43%.

On the basis of the odds ratio of failure associated with a risk factor, we assigned five risk points to severe or severe-complicated CDI, four risk points to inpatient setting, and one point to each CDI-related hospitalization before FMT (**Table 2**). The risk score for a patient is calculated by adding the points for each risk factor. For example, a patient with severe CDI and two CDI-related hospitalizations before FMT would have a risk score of 7 points if the FMT is performed in an outpatient setting. The risk scores ranged from 0 to 13 points in the developmental cohort, with a mean of 2.5 and a standard deviation of 3.3. Tertiles of the risk scores were used to divide patients equally into low-, moderate-, and high-risk groups. In the developmental cohort, patients in the low-risk category (0 points) had a 5.6% failure rate; patients in the moderate-risk group (1–2 points) had a 12.7% failure rate; and patients in the high-risk category (3 points or higher) had a 41% failure rate (**Table 3**).

The validation cohort included a total of 134 patients (**Table 4**). The overall failure rate was 22.4% (19.4% for early failure and 3% for late failure). Similar to the developmental cohort, the risk scoring system discriminated well among the three risk categories at predicting early FMT failure, with failure rates for low-, moderate-,

Table 2. Risk factors associated with FMT failure at 1 month after treatment in multivariable analysis in the developmental cohort and associated risk points

Risk factor	Odds ratio (95% confidence interval)	*P* value	Estimated risk points
Severe or severe/complicated indication	5.95 (2.26–15.62)	<0.001	5
Number of CDI-related hospitalization before FMT	1.43 (1.18–1.75)	<0.001	1
Inpatient FMT	3.78 (1.55–9.24)	0.004	4

CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation.

Table 3. Rates of early FMT failure according to risk scores in the developmental and validation cohorts

Risk score	Developmental cohort		Validation cohort	
	N	FMT failure	*N*	FMT failure
0	126	7 (5.6%)	47	1 (2.1%)
1–2	102	13 (12.7%)	31	5 (16.1%)
3+	100	41 (41%)	56	20 (35.7%)
Area under the ROC curve	0.81		0.84	

FMT, fecal microbiota transplantation; ROC, receiver operating characteristic.

and high-risk groups at 2.1, 16.1, and 35.7%, respectively. (**Table 3**) The point scoring system had slightly better discrimination in the validation cohort than in the developmental cohort, with an AUROC for FMT failure of 0.81 in the developmental cohort and 0.84 in the validation cohort (**Figure 1**).

As inpatient status is likely surrogate for patients' overall disease severity and health status, we developed a second risk scoring model without the inpatient variable. Excluding inpatient status did not identify factors predictive of early FMT failure in addition to severe or severe-complicated CDI and a number of CDI-related hospitalizations before FMT. On the basis of the odds ratios estimated in this model, we assigned eight risk points to severe or severe-complicated CDI (OR 14.48, 95% CI 6.82–30.7, $P<0.001$) and one risk point to the each CDI-related prior hospitalization (OR 1.41, 95% CI 1.16–1.72, $P<0.001$). Early failure rates for each risk tertile in the developmental and validation cohorts are shown in **Table 5** and receiver operating characteristic curves in **Figure 2**. Prediction models without the inpatient status yielded slightly worse discrimination in the developmental (1% lower AUROC) and validation cohorts (3% lower AUROC).

As the majority of patients who underwent FMT were outpatients, we performed a subgroup analysis to identify risk factors of FMT failure among outpatient FMTs. Data from all three sites were combined for this analysis because of the small number of early failures ($N=37$ failures). On the basis of the multivariable logistic regression model, we found a single predictor of early FMT failure: each CDI-related hospitalization before FMT increased the risk by 1.4 fold (95% CI: 1.16–1.69, $P<0.001$, risk point=1). Failure rates according to risk scores ranging from 0 to 8 are depicted in **Table 6**.

Only 2.8% ($N=13$) of patients in the developmental and validation cohort combined ($N=462$) had late failure between 1 and 3 months. Of these, 53.8% ($N=7$) were females, 46.2% ($N=6$) had IBD on immunosuppressive therapy, and 92.3% ($N=12$) had recurrent CDI indication for FMT.

Table 4. Baseline characteristics of the validation cohort at the time of FMT

	Total (*N*=134)	Success at 1 month (*N*=108)	Failure at 1 month (*N*=26)
Patient characteristics			
Age, mean (s.d.)	65.89 (18.07)	64.74 (18.74)	70.68 (14.27)
Female gender, *n* (%)	77 (57.5%)	64 (59.3%)	13 (50.0%)
IBD, *n* (%)	13 (9.7%)	12 (11.1%)	1 (3.8%)
Crohn's disease, *n* (%)	6 (4.5%)	5 (4.6%)	1 (3.8%)
Ulcerative colitis, *n* (%)	7 (5.2%)	7 (6.5%)	0 (0%)
Immunocompromised state, *n* (%)	24 (17.9%)	15 (13.9%)	9 (34.6%)
Anti-secretory therapy before FMT, *n* (%)	70 (52.2%)	53 (49.1%)	17 (65.4%)
CDI history and management			
Presence of CDI-related hospitalization before FMT, *n* (%)	87 (64.9%)	62 (57.4%)	25 (96.2%)
Number of CDI-related hospitalization before FMT, mean (s.d.)	1.49 (1.58)	1.39 (1.65)	1.88 (1.21)
Use of CDI antibiotics within 8 weeks of FMT, *n* (%)	134 (100%)	108 (100%)	26 (100%)
Presence of pseudomembranes at FMT, *n* (%)	8 (6.0%)	0 (0%)	8 (30.8%)
Inpatient FMT, *n* (%)	40 (29.9%)	21 (19.4%)	19 (73.1%)
WBC at FMT, mean (s.d.)	8.03 (3.89)	7.61 (3.52)	9.69 (4.83)
FMT			
FMT indication, *n* (%)			
Recurrent	117 (87.3%)	105 (97.2%)	12 (46.2%)
Severe	15 (11.2%)	3 (2.78%)	12 (46.2%)
Severe/Complicated	2 (1.5%)	0 (0%)	2 (7.6)
Donor type: patient directed, *n* (%)	13 (9.7%)	12 (11.1%)	1 (3.8%)

CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation; IBD, inflammatory bowel disease; WBC, white blood cell count.

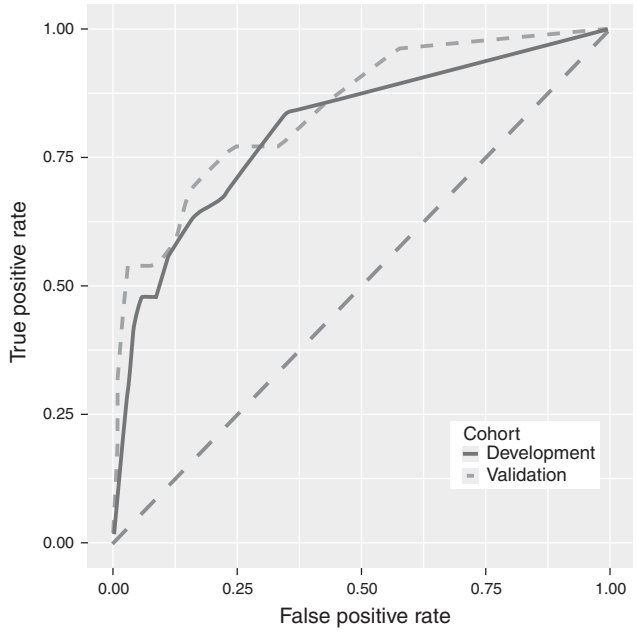

Figure 1. Receiver operating characteristic (ROC) curves for the prediction of fecal microbiota transplantation (FMT) failure at 1 month using risk scores.

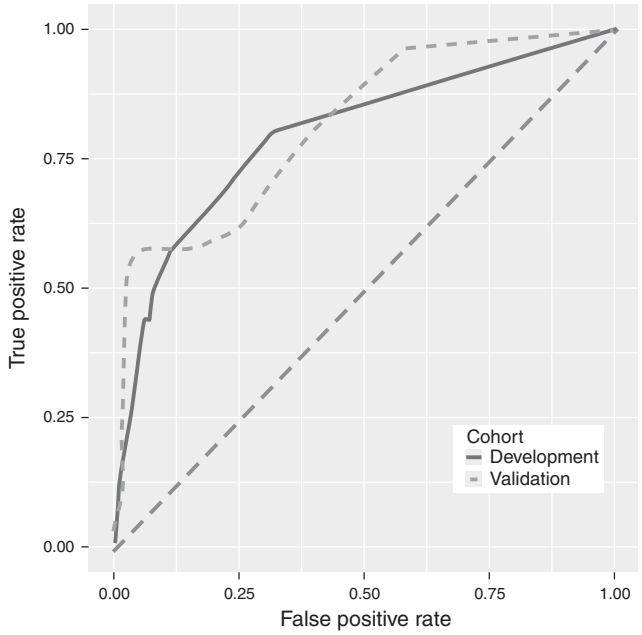

Figure 2. Receiver operating characteristic (ROC) curves for the prediction of fecal microbiota transplantation (FMT) failure at 1 month using risk scores with inpatient FMT excluded from the model.

Table 5. Rates of early FMT failure according to risk scores in the developmental and validation cohorts based on the model without inpatient variable

Risk score	Developmental cohort		Validation cohort	
	N	FMT failure	*N*	FMT failure
0	133	8 (6%)	47	1 (2.1%)
1–2	109	14 (12.8%)	44	9 (20.5%)
3+	86	39 (45.4%)	43	16 (37.2%)
Area under the ROC curve	0.80		0.81	

FMT, fecal microbiota transplantation; ROC, receiver operating characteristic.

Table 6. Rates of early FMT failure according to risk score (the number of CDI-related hospitalizations before FMT) in the combined developmental and validation cohort in the outpatient setting

Risk score	*N*	FMT failure
0	173	9 (5.2%)
1–2	136	18 (13.2%)
3+	55	10 (18.2%)
Area under the ROC curve	0.68	

CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation; ROC, receiver operating characteristic.

DISCUSSION

FMT is the most efficacious treatment for recurrent or therapy-refractory CDI; however, in 10–20% of cases, a single FMT fails to provide cure. The reason for this is unclear, although it has been speculated that patient and CDI disease characteristics or procedural methods may all be factors. To our knowledge, this large multicenter, retrospective study is the first to describe clinical variables associated with failed FMT and to develop and validate a risk model to predict FMT failure.

We found that the majority of FMT failure following a single treatment occurred within the first month (early failure). Only a small percentage of FMT failure occurred between 1 and 3 months post FMT (late failure). We have identified several independent predictors of early failure: severity of CDI (OR=5.95), the need of inpatient FMT (OR=3.78), and a number of CDI-associated hospital admissions (OR=1.43). As inpatient FMT is likely a surrogate for patients' overall disease severity and health status, we have built a risk prediction model using either all three variables, each assigned a different weight based on OR, or excluding inpatient FMT as a factor. The prediction model with inpatient factor included performed slightly better in discriminating between low-risk and high-risk FMT failure in both developmental and validation cohorts.

Intuitively, it makes sense that severe CDI and the need to perform FMT as an inpatient could predict FMT outcomes. Previous studies have found that a single FMT was not enough to cure severe CDI. For example, Weingarden *et al.* (18) reported that

sequential FMT was needed for the resolution of severe CDI in two of four patients. In a randomized trial by Cammarota *et al.* (6) comparing FMT with vancomycin for CDI, the first two patients with pseudomembranous colitis, a marker of disease severity, died after receiving only a single FMT. Therefore, the authors changed their protocol, offering multiple FMTs to those with severe disease, curing all five subsequent patients with pseudomembranous colitis. More recently, Fischer *et al.* (15) published a sequential FMT protocol, using FMT in conjunction with vancomycin for severe and severe-complicated CDI based on the presence or absence of pseudomembranes. In this study, 13 of 29 patients with severe and/or complicated CDI required 2 sequential FMTs within 10 days, with an additional 2 patients requiring a third FMT in the course of their management.

Regardless of CDI severity, patients who undergo FMT while hospitalized are at a greater risk for ongoing exposure to the organism in the health-care environment and treatment with antibiotics for other infections, factors that may contribute to FMT failure. Indeed, we found that patients with a greater number of CDI-related hospitalizations before FMT are more likely to have a failure outcome. With every additional hospitalization, the odds of failure increase by 43%. These findings are in keeping with those of Baggs *et al.,* (19) who found that the number of hospitalizations in the prior 90 days was predictive of CDI recurrence. It is possible that increased exposure to the health-care environment itself has a deleterious effect on the distal gut microbiota, putting patients at greater risk of recurrence. Alternatively, hospitalization may just represent a risk for antibiotic exposure.

Interestingly, the presence of immunosuppression and IBD were not found to be predictors of early FMT failure in this study, although both of these factors are known to be risk factors of CDI (20–22). We previously reported that the success rate after a single FMT was 78% in a multicenter study involving 80 immunocompromised patients, a rate similar to that seen in immunocompetent patients (16). In the subset of 36 IBD patients, 86% achieved CDI resolution. Because of the small number of patients it was not possible to determine whether IBD was a factor affecting FMT outcome. More recently, Khoruts *et al.* (23) found that IBD had a negative impact on FMT outcome, in that patients with concurrent IBD and CDI (N=43) had only 74% chance of clearing CDI with a single colonoscopic FMT compared with 92% in those without IBD (N=229, P=0.0018). However, it was not known whether these IBD patients had active disease at the time of FMT. In their study, immunosuppressive therapy did not impact the FMT outcome. Both of these prior studies defined FMT failure at 2–3 months rather than 1 month as was done in our study. Immunosuppression and IBD might have a different impact on early vs. late failure. Given the small numbers of patients in these retrospective studies, no definitive conclusions can yet be drawn.

The strengths of this study relate to its large sample size and multicenter nature. Each of the study centers has extensive clinical and research interest in FMT, following treated patients closely with telephone calls and clinic visits and maintaining a database of outcomes. The multicenter nature of our study allowed for both the development and validation of a prediction model. The variables incorporated in this model made clinical sense and were shown to differ significantly between those who succeeded or failed the first FMT. This simple and yet novel prediction model appeared to have good discriminative power at identifying individuals who are at high risk of failure after FMT therapy and may assist the treating physician in subsequent management plans.

The weaknesses here include inherent limitations of retrospective studies, such as selection bias and documentation errors. Not all variables collected in the developmental cohort were collected in the validation cohort. Some of the data such as antibiotic exposure post FMT were recorded retrospectively and were incomplete as patients may have received subsequent care outside the study centers. Therefore, the effect of post-FMT antibiotic exposure on the failure rate could not be examined. As FMT was administered predominantly by lower endoscopy in all three centers, it was not possible to determine whether the route of delivery has an effect on failure rates. Furthermore, the weight of donor stool was not examined as a contributor to failure, which had been shown in a systematic review to be a potential factor (11). In addition, ribotyping of *Clostridium difficile* was not performed on these patients, as this is not standard practice, nor widely available, and therefore whether the hypervirulent strains such as 027/B1/NAP1 may be another potential variable cannot be determined. Lack of typing also prevented our ability to distinguish between relapse with the original strain vs. reinfection with a different strain.

Studying the fecal microbiome in patients who have failed multiple FMTs may reveal persistent dysbiosis or signature communities predisposing to ongoing CDI. Understanding keystone species associated with successful FMT may help optimize donor selection and guide the development of next-generation microbial therapeutics. A national registry of FMT patients, with clinical data and outcomes, would access real-world effectiveness and safety of the intervention and promote scientific investigation around manipulation of gut microbiota in humans. Finally, a prospective clinical trial offering sequential FMTs at baseline for patients at high risk for failure would help determine the efficacy of the intervention.

In conclusion, this is the first study to describe risk factors associated with FMT failure and to develop a risk score to predict patients at high risk of failing a first FMT. This simple scoring system may be a valuable tool for clinicians as they make decisions and counsel patients around the utility of FMT in various clinical situations.

CONFLICT OF INTEREST

Guarantor of the article: Monika Fischer, MD, MSc.
Specific author contributions: Study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content: Monika Fischer, Dina Kao, and Colleen R. Kelly; acquisition of data: Shama R. Mehta, Tracey Martin, Gwendolyn K. Cook, and Emmalee Phelps; acquisition of data, analysis and interpretation of data: Joseph Dimitry and Ammar H. Keshteli; study concept, drafting the manuscript, and critical revision of

the manuscript for important intellectual content: Brian W. Sipe; analysis and interpretation of data, drafting the manuscript, and critical revision of the manuscript for important intellectual content: Huiping Xu.

Financial support: Dr Kelly was funded by NIH/NIDDK 1R21DK093839-01A and Dr Kao by Alberta Health Services and University of Alberta Hospital Foundation. Joseph Dimitry was supported by the Canadian Association of Gastroenterology Summer Studentship. Statistical analysis by Dr Xu was supported by the ASGE Covidien Senior Investigator Mentoring Award to Thomas F. Imperiale, Indiana University.

Potential competing interests: Dr Kelly served as a consultant and site investigator for Seres Health and received research support from Assembly Biosciences. Dr Fischer served as a consultant for Rebiotix, and Dr Kao received research support from Rebiotix.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Fecal microbiota transplant (FMT) is a highly efficacious treatment for recurrent or therapy refractory *Clostridium difficile* infection (CDI).
- ✓ A total of 10–20% of patients fail to achieve cure after a single FMT.

WHAT IS NEW HERE

- ✓ The majority of failures occur early, within 1 month of FMT.
- ✓ Inpatient status during FMT, severe or severe-complicated CDI, and previous CDI-related hospitalizations are strongly associated with early FMT failure.
- ✓ In the outpatient setting, previous CDI-related hospitalizations increase the risk of FMT failure.
- ✓ The proposed simple risk index has good discriminative power at identifying individuals at high risk of failure after FMT therapy and may assist the clinicians in subsequent management plans.

REFERENCES

1. McFarland LV, Elmer GW, Surawicz CM. Breaking the cycle: treatment strategies for 163 cases of recurrent Clostridium difficile disease. The Am J Gastroenterol 2002;97:1769–75.
2. Mattila E, Uusitalo-Seppala R, Wuorela M *et al.* Fecal transplantation, through colonoscopy, is effective therapy for recurrent Clostridium difficile infection. Gastroenterology 2012;142:490–6.
3. Bakken JS, Borody T, Brandt LJ *et al.* Treating Clostridium difficile infection with fecal microbiota transplantation. Clin Gastroenterol Hepatol 2011;9:1044–9.
4. Borody TJ, Paramsothy S, Agrawal G. Fecal microbiota transplantation: indications, methods, evidence, and future directions. Curr Gastroenterol Rep 2013;15:337.
5. Kelly CR BL, Abd M, Alani M *et al.* A multicenter, randomized, placebo-controlled, double-blind study to evaluate the efficacy and safety of fecal microbiota transplantation in patients with recurrent Clostridium difficile infection. American College of Gastroenterology Meeting, Honolulu, HI, 2015
6. Cammarota G, Masucci L, Ianiro G *et al.* Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection. Aliment Pharmacol Ther 2015;41:835–43.
7. van Nood E, Dijkgraaf MG, Keller JJ. Duodenal infusion of feces for recurrent Clostridium difficile. N Engl J Med 2013;368:2145.
8. Kassam Z, Lee CH, Yuan Y *et al.* Navigating long-term safety in fecal microbiota transplantation. Am J Gastroenterol 2013;108:1538.
9. Gough E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. Clin Infect Dis 2011;53:994–1002.
10. Cammarota G, Ianiro G, Bibbo S *et al.* Fecal microbiota transplantation: a new old kid on the block for the management of gut microbiota-related disease. J Clin Gastroenterol 2014;48(Suppl 1):S80–4.
11. Kassam Z, Lee CH, Yuan Y *et al.* Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. Am J Gastroenterol 2013;108:500–8.
12. Hamilton MJ, Weingarden AR, Sadowsky MJ *et al.* Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. Am J Gastroenterol 2012;107:761–7.
13. Lee CH, Steiner T, Petrof EO *et al.* Frozen vs. fresh fecal microbiota transplantation and clinical resolution of diarrhea in patients with recurrent Clostridium difficile infection: a randomized clinical trial. JAMA 2016;315:142–9.
14. Lee CH, Belanger JE, Kassam Z *et al.* The outcome and long-term follow-up of 94 patients with recurrent and refractory Clostridium difficile infection using single to multiple fecal microbiota transplantation via retention enema. Eur J Clin Microbiol Infect Dis 2014;33:1425–8.
15. Fischer M, Sipe BW, Rogers NA *et al.* Faecal microbiota transplantation plus selected use of vancomycin for severe-complicated Clostridium difficile infection: description of a protocol with high success rate. Aliment Pharmacol Ther 2015;42:470–6.
16. Kelly CR, Ihunnah C, Fischer M *et al.* Fecal microbiota transplant for treatment of Clostridium difficile infection in immunocompromised patients. Am J Gastroenterol 2014;109:1065–71.
17. Surawicz CM, Brandt LJ, Binion DG *et al.* Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J Gastroenterol 2013;108:478–98;quiz 99.
18. Weingarden AR, Hamilton MJ, Sadowsky MJ *et al.* Resolution of severe Clostridium difficile infection following sequential fecal microbiota transplantation. J Clin Gastroenterol 2013;47:735–7.
19. Baggs J, Yousey-Hindes K, Ashley ED *et al.* Identification of population at risk for future Clostridium difficile infection following hospital discharge to be targeted for vaccine trials. Vaccine 2015;33:6241–9.
20. Ananthakrishnan AN, McGinley EL, Saeian K *et al.* Temporal trends in disease outcomes related to Clostridium difficile infection in patients with inflammatory bowel disease. Inflamm Bowel Dis 2011;17:976–83.
21. Sanchez TH, Brooks JT, Sullivan PS *et al.* Bacterial diarrhea in persons with HIV infection, United States, 1992–2002. Clin Infect Dis 2005;41:1621–7.
22. Gorschluter M, Glasmacher A, Hahn C *et al.* Clostridium difficile infection in patients with neutropenia. Clin Infect Dis 2001;33:786–91.
23. Khoruts A, Rank KM, Newman KM *et al.* Inflammatory bowel disease affects the outcome of fecal microbiota transplantation for recurrent Clostridium difficile infection. Clin Gastroenterol Hepatol 2016 (in press).