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Randomised clinical trial: faecal microbiota transplantation by colonoscopy plus vancomycin for the treatment of severe refractory *Clostridium difficile* infection—single versus multiple infusions

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Summary

Background: Faecal microbiota transplantation (FMT) is a highly effective treatment against recurrent *Clostridium difficile* infection. Far less evidence exists on the efficacy of FMT in treating severe *Clostridium difficile* infection refractory to antibiotics.

Aim: To compare the efficacy of two FMT-based protocols associated with vancomycin in curing subjects with severe *Clostridium difficile* infection refractory to antibiotics.

Methods: Subjects with severe *Clostridium difficile* infection refractory to antibiotics were randomly assigned to one of the two following treatment arms: (1) FMT-S, including a single faecal infusion via colonoscopy followed by a 14-day vancomycin course, (2) FMT-M, including multiple faecal infusions plus a 14-day vancomycin course. In the FMT-M group, all subjects received at least two infusions, while those with pseudomembranous colitis underwent further infusions until the disappearance of pseudomembranes. The primary outcome was the cure of refractory severe *Clostridium difficile* infection.

Results: Fifty six subjects, 28 in each treatment arm, were enrolled. Twenty one patients in the FMT-S group and 28 patients in the FMT-M group were cured (75% vs 100%, respectively, both in per protocol and intention-to-treat analyses; $P = 0.01$). No serious adverse events associated with any of the two treatment protocols were observed.

Conclusions: A pseudomembrane-driven FMT protocol consisting of multiple faecal infusions and concomitant vancomycin was significantly more effective than a single faecal transplant followed by vancomycin in curing severe *Clostridium difficile* infection refractory to antibiotics. Clinical-Trials.gov registration number: NCT03427229.

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1 | INTRODUCTION

Clostridium difficile (now called *Clostridioides difficile*) infection (CDI) is the most common hospital-acquired cause of diarrhoea, and has become a major challenge for healthcare systems, accounting for nearly $5 billion in healthcare cost[1,2] and 29 000 deaths[3] per year in the United States. The burden of CDI in the last decade can be explained mostly by the increase in incidence, severity, mortality, and likelihood of recurrence.[3] Most recent data show that nearly 20% of patients with newly diagnosed CDI recur after standard antibiotic therapy, and recurrence rates rise up to 50%-60% after the second recurrence.[4,5]

Due to its resistance to antibiotics, recurrent CDI is more likely to present with a severe clinical picture, which increases the risk of life-threatening complications (ie toxic megacolon, sepsis) and death.[6]

Faecal microbiota transplantation (FMT) is a highly effective and durable therapy for recurrent CDI,[7-10] and it is recommended as the best therapeutic option for recurrent disease after failure of antibiotics.[11-14] Evidence suggests that FMT may be a promising treatment also for severe CDI refractory to antibiotics, as reported cure rates range from 50% to 91%.[15-21] Due to high morbidity and mortality associated with colectomy,[22] the use of FMT has been recommended in this subset of patients,[13] although there is still considerable uncertainty about the best therapeutic protocol to adopt.

As reported in most studies, single-infusion FMT is likely to provide only transient improvement in patients with severe CDI and pseudomembranous colitis, and multiple infusions are often necessary to obtain sustained cure.[17-20] In our early experience, we administered repeated faecal infusions to patients with pseudomembranous colitis until the disappearance of pseudomembranes, achieving a 100% cure rate in patients treated with this approach.[8] More recently, Fischer and colleagues described a specific protocol for severe and complicated CDI including an initial faecal infusion in all patients, further vancomycin treatment in those with pseudomembranes, and repeated FMT in nonresponders.[18,20] In their largest report, the authors achieved nearly 53% and 96% efficacy rates with single and repeated faecal infusions, respectively.[20] In two other cohort studies,[17,23] multivariate analysis found severe CDI to be an independent predictor of failure after single faecal infusion.

Although preliminary data is promising, the definition of an effective FMT protocol for severe CDI is limited by the absence of randomised trials comparing single versus multiple infusions. Accordingly, we aimed to compare the efficacy of two different FMT protocols including, respectively, single or multiple faecal infusions plus vancomycin for the treatment of severe CDI refractory to antibiotics.

2 | METHODS

2.1 | Study design

In this open-label, randomised clinical trial we compared the two following experimental treatments in subjects with severe CDI refractory to antibiotics: *FMT-S*), including a single infusion of faeces from healthy donor plus a 14-day vancomycin course; *FMT-M*), including multiple (at least two) faecal infusions from healthy donor plus a 14-day vancomycin course.

The study was performed at the Fondazione Policlinico Universitario "A. Gemelli" in Rome and carried out following Consolidated Standards of Reporting Trials (CONSORT) Statement.[24] Referred subjects were evaluated by G. C. and G. I. to determine their eligibility for the study. All enrolled subjects provided their written informed consent. The study protocol was approved by the local ethics committee.

2.2 | Criteria for inclusion

Patients who were at least 18 years of age, had a life expectancy ≥3 months, and severe CDI refractory to one or more courses of specific antibiotic therapy (≥10 days of vancomycin, at a dosage of ≥250 mg four times daily; or ≥10 days of fidaxomycin at dosage of 200 mg two times a day) were considered for inclusion.

We excluded patients with bowel perforation, toxic megacolon and septic shock. Other exclusion criteria included: subjects <18 years old; prior colectomy, colostomy or ileostomy; ongoing treatment for malignancy; concomitant therapy with systemic antibiotics; mild clinical picture of CDI; high risk of colonoscopy complications; other relevant gastrointestinal diseases (e.g. Crohn's disease or ulcerative colitis) or other infectious causes of diarrhoea beyond CDI; human immunodeficiency virus (HIV) 1-2; pregnancy or breastfeeding; inability to follow protocol procedures; patients who were not able to give or refused to sign consent.

2.3 | Definitions

CDI was defined as a clinical picture compatible with CDI and microbiological evidence of free toxins and the presence of *C. difficile* in stool without reasonable evidence of another cause of diarrhoea, or pseudomembranous colitis as diagnosed during endoscopy, after colectomy or on autopsy.[12] Mild CDI was defined as diarrhoea as the only symptom.[11] Severe CDI was defined, following ESCMID guidelines,[12] as an episode of CDI with at least one specific sign or symptom of severe colitis, or a complicated course of disease (significant systemic toxin effects and shock that result in need for admission to intensive care unit, colectomy or death). Signs and symptoms of severe colitis included clinical (fever, haemodynamic instability, respiratory failure which needs mechanical ventilation, signs and symptoms of peritonitis, signs and symptoms of colonic ileus), laboratory (marked leukocytosis, rise in serum creatinine and lactate, marked decrease in serum albumin), radiological (colon distension, colonic wall thickening) or endoscopic (presence of pseudomembranes) markers.

Severe-complicated CDI was defined, according to the ACG guidelines,[11] as disease presenting with or developing one or more of the following signs or symptoms: admission to intensive care unit, hypotension with or without required use of vasopressors, fever ≥ 38.5°C, ileus (symptoms including nausea, emesis, sudden

interruption of diarrhoea, abdomen distention or radiological signs suggesting altered intestinal transit), or significant abdominal distention, mental status changes, white blood cells ≥35 000 cells/mm^3 or <2000 cells/mm^3, serum lactate levels >2.2 mmol/L or any evidence of end organ failure.

Refractory CDI was defined as CDI not responsive to antibiotics, or rather persistent diarrhoea with positive *C. difficile* toxin in the absence of other possible causes of diarrhoea.

Finally, recurrent CDI was defined as diarrhoea (at least 3 loose or watery stools per day for 2 or more consecutive days, or at least eight loose stools in 48 hours) unexplainable by other causes, with or without positive stool toxin within 8 weeks from the end of the therapy. These definitions were defined following international guidelines.[12]

2.4 | Study treatments

Patients were randomly assigned to one of the following treatment arms:

1. *FMT-S)*, including a 3-day pre-treatment with vancomycin (250 mg by mouth four times a day), followed by a single faecal infusion and then by a 14-day vancomycin course (250 mg by mouth four times per day) started the day after the procedure;
2. *FMT-M)*, including a 3-day pre-treatment with vancomycin (250 mg by mouth four times per day), followed by at least two faecal infusions administered every 3 days and associated with a 14-day vancomycin course (250 mg by mouth four times per day) started the day after the first infusion. Patients with pseudomembranous colitis received further faecal infusions every 3 days until the disappearance of pseudomembranes.

The timeline of the scheduled treatments is detailed in Figure 1.

Patients who were not cured after any of the two scheduled treatments were offered off-protocol therapy with further FMT.

2.5 | Selection of donors

The selection of donors was performed by two authors (G. C. and G. I.) following protocols previously recommended by international guidelines, including: a questionnaire to address donor medical history; blood and stool exams to exclude potentially transmittable diseases (Table S1); and a further questionnaire administered to selected donors the day of the faeces collection to rule out any issue happened within the screening period.[13] The assignment of faecal infusates from healthy donors to patients was done randomly, without any specific recipient-donor match, as suggested by international guidelines.[13]

2.6 | Manufacturing of faecal infusate

All faecal infusate samples were prepared in the microbiology laboratory of our hospital, using either fresh or frozen faeces, using at least 50 grams of faeces for each sample. We followed manufacturing protocols recommended by international guidelines for fresh and frozen faeces, respectively.[13] Frozen infusate samples were stored at −80°C.

2.7 | Faecal infusion procedure

All procedures were performed by colonoscopy. All patients underwent bowel cleansing with 2 litres of macrogol (SELG ESSE) per day for 2 days before the first procedure. Patients in the FMT-M arm were also restricted to a light diet and underwent a restricted bowel preparation (2 litres of macrogol) every 3 days before further infusions.

All procedures were performed by 3 expert endoscopists (G. C., G. I., L. R. L.), using paediatric colonoscopes and carbon dioxide insufflation. The infusate was delivered within 6 hours after donor supply (if fresh faeces were used) or after thawing (if frozen faeces were used), through the operative channel of the scope after reaching the more proximal point of the large bowel, using 50 mL syringes filled with the infusate during colonoscopy. During the insertion and removal of the colonoscope, operators were able to assess the presence of pseudomembranes and other inflammatory signs of the large bowel. Finally, the patients were monitored in the recovery room of the endoscopy centre for 2-3 hours after the procedure.

2.8 | Outcomes and follow-up

The primary outcome was the cure of refractory severe CDI. We defined the cure of refractory severe CDI as the progressive reduction in diarrhoea and improvement of clinical picture within 1 week after any of the scheduled treatment, and the disappearance of diarrhoea, or persistent diarrhoea explicable by other causes, 8 weeks after any of the scheduled treatments.

The secondary outcome was the occurrence of serious adverse events (defined as death or life-threatening conditions, for example, bowel perforation or bloodstream infections) within 8 weeks after any of the scheduled treatments.

Patients were closely followed up by the four authors (C. S., G. C., G. I., L. R. L) in the days after treatment, and a stool diary was kept by the patients themselves, by family members, or by referral physicians or nurses. They were also asked about stool frequency and consistency, drug use and adverse events up to 8 weeks after the end of treatment. Patients in the FMT-S group were followed up every day for 1 week after the FMT, and then every week after the end of vancomycin course, up to 8 weeks later. Patients in the FMT-M group were followed up every day between faecal infusions and for 1 week after the last infusion, and then every week after the end of vancomycin course, up to 8 weeks later. Stool tests for *C. difficile* toxin were performed at week 8 and whenever diarrhoea occurred, using a Premier Toxins A&B (Liaison® *C.difficile* GDH-Toxin A/B – DiaSorin Inc. 1951 Northwestern Avenue Stillwater. MN, USA) kit in the microbiology laboratory of the hospital.

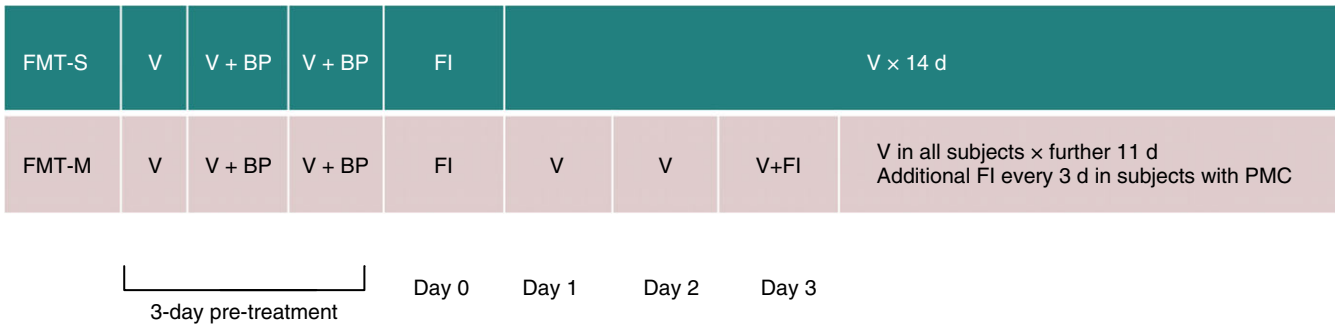

FIGURE 1 Timeline of the scheduled treatments after randomisation. BP, Bowel preparation (2 litres of macrogol); FI, faecal infusion; FMT-M, multiple-infusion treatment arm; FMT-S, single-infusion treatment arm; PMC, Pseudomembranous colitis; V, vancomycin (250 mg four times a day by mouth)

2.9 | Randomisation

Blocked randomisation of subjects was performed by an external individual not involved in the study. An online random number generator software (https://www.sealedenvelope.com/simple-randomiser/v1/lists) was used to provide random permuted blocks with a block size of six and an equal allocation ratio; the sequence was concealed until the interventions were assigned. Because of the intrinsic difference between the two treatments, neither physicians nor patients were blinded to the randomisation groups.

2.10 | Statistical analysis

Calculation of sample size was based on the superiority of multiple faecal infusions vs single faecal infusion via colonoscopy in curing severe refractory CDI (respectively, 90% vs 30%) previously reported by our research team.[17] As a 14-day vancomycin regimen was added to both FMT treatment arms, a cure rate of 90% for FMT-M and of 50% for FMT-S in treating severe refractory CDI were assumed. With a two-tailed α value of 0.05 and a power of 90% ($\beta = 0.10$), the enrolment of 26 patients per group was required. Sample size was calculated with an online software (http://www.stat.ubc.ca/~rollin/stats/ssize/b2.html). On the basis of our previous experience,[8] we considered a 7%-10% of potential dropouts, so we planned to enrol 28 patients per group.

Analyses were performed both on an intention-to-treat and per-protocol basis. Differences among groups were assessed with Student's *t* test for continuous data and with Fisher's exact probability test (using two-tailed *P*-values) for categorical data. Differences in cure percentages were determined with Fisher's exact test (with two-tailed *P*-values). Statistical analyses were carried out with an online calculator (http://www.graphpad.com/quickcalcs/) and with Microsoft Excel for Mac (Microsoft Excel. Redmond, Washington: Microsoft, 2011).

3 | RESULTS

3.1 | Patient characteristics

From January 2016 through November 2017, 68 subjects with refractory CDI were assessed for eligibility. Twelve subjects were excluded for the following reasons: five subjects for absence of criteria for severe CDI; one for underlying IBD; one due to stool positivity for other pathogens beyond *C. difficile*; two for concomitant therapy with systemic antibiotics; one for septic shock and two for toxic megacolon. The remaining 56 subjects (F = 39, M = 17, mean age 75 years) were randomly assigned to one of the two following treatment arms: FMT-S (28 subjects, F = 18, M = 10, mean age: 75 years) or FMT-M (28 subjects, F = 21, M = 7, mean age 74 years). All patients accepted the proposed treatment. The participants' flow diagram is detailed in Figure 2.

All 56 enrolled subjects were in-patients and suffered from severe CDI refractory to standard antibiotic regimens. All included patients had positive stool testing for *C. difficile* toxin at enrolment.

Twenty-four patients in the FMT-S group and 25 patients in the FMT-M group, respectively, had recurrent disease, while others (seven subjects, 12%) were experiencing their first episode of CDI. Thirty-three subjects (59%-17 in the FMT-S group and 16 in the FMT-M group, respectively) had severe-complicated CDI, as defined by Surawicz and colleagues.[11] Pseudomembranous colitis was observed in 36 patients (64%-17 in the FMT-S group and 19 patients in the FMT-M group).

There were no significant differences in demographic and clinical characteristics between the two groups at baseline (Table 1).

3.2 | Donor and FMT characteristics

Fifty-two potential donors were screened for eligibility in the study period. Twenty of them were excluded for the following reasons: recent (<3 months) antibiotic treatment (n = 9); chronic therapy with proton pump inhibitors (n = 5); high-risk sexual behaviour (n = 1); high serum aminotransferase levels (n = 2); stool testing positive for *Blastocystis hominis* (n = 3). Therefore, faeces from a total of 32 donors were used to treat 56 subjects in both groups; a mean ($\pm$ SD) of 74 $\pm$ 22 g of faeces were infused. The mean time from defecation to infusion was 4.5 $\pm$ 1.2 hours.

In the FMT-S group, 20 patients received faeces from unrelated donors (71%) and 8 from related donors (29%), while 12 patients (43%) were treated with frozen faeces and 16 with fresh faeces (57%). Each subject in the FMT-M group received three faecal

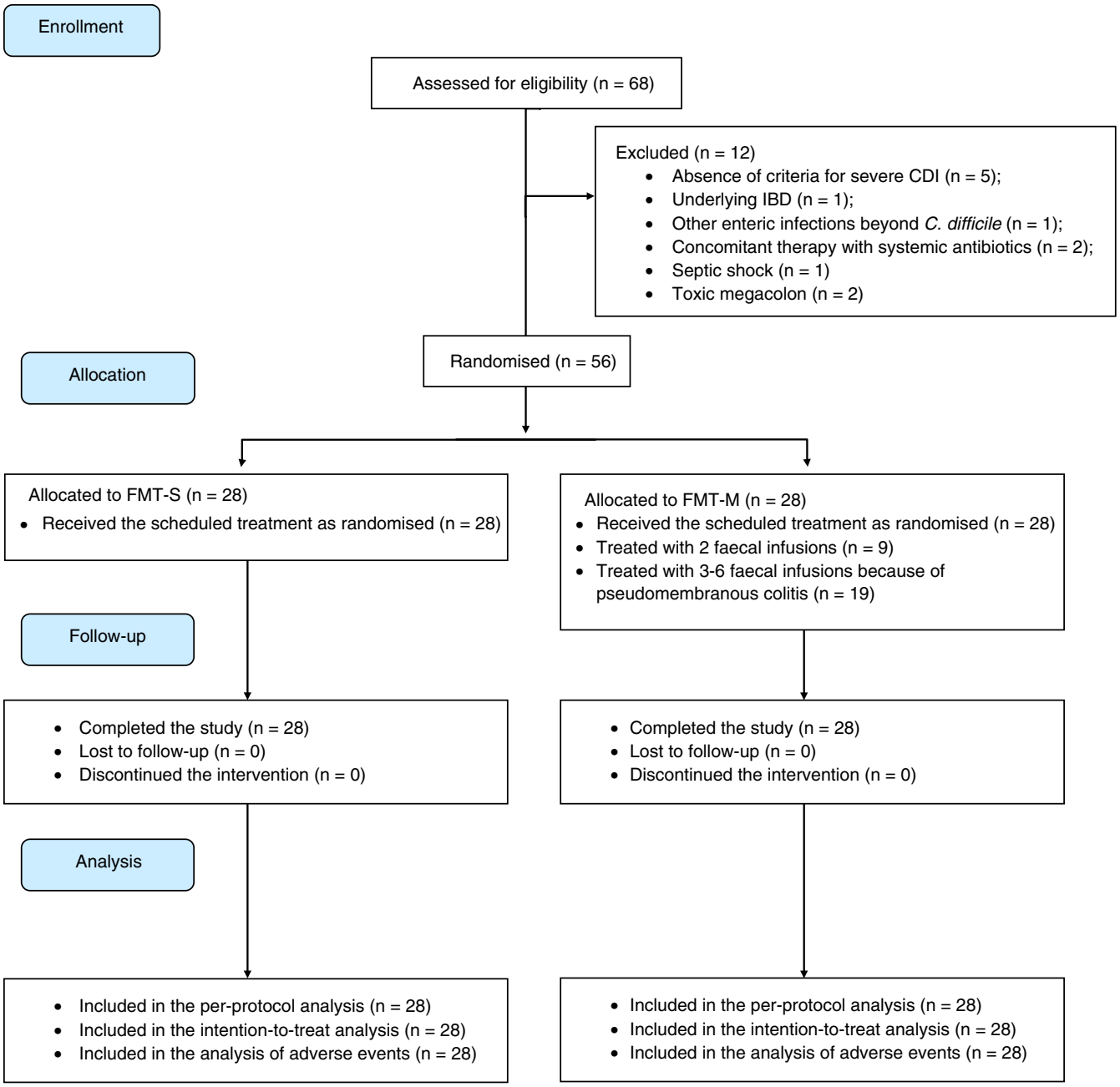

FIGURE 2 Flow diagram of the subjects enrolled in the study

infusions on average (range 2-6), in a total of 89 infusions. For each infusion, subjects received stools from the same donor or from different donors, depending on availability. Fresh faeces were used in 50 infusions (56%) and frozen faeces in 39 infusions (44%); faeces from unrelated donors were used for 63 procedures (71%) and related donors were used for 26 procedures (29%).

3.3 | Study outcomes

In the FMT-S group, 21 of the 28 patients were cured (75% both in per-protocol and intention-to-treat analysis); ten of them had pseudomembranous colitis, and 13 had severe-complicated CDI. Of the seven failing subjects, all had pseudomembranous colitis, and four presented with severe-complicated CDI. A transient clinical improvement was observed immediately after FMT in all of them, but they experienced a relapse of *C. difficile*-associated diarrhoea and a worsening of their clinical picture on average 10 days after the end of treatment (range 7-19 days). All 7 subjects received off-protocol treatment with further faecal infusions and were ultimately cured from CDI; however, one patient died 1 month after the end the off-protocol faecal infusions of heart attack.

In the FMT-M group, all 28 patients (100%, on both per-protocol and intention-to-treat analyses) started ameliorating immediately

TABLE 1 Baseline demographic and clinical characteristics of the patients

Characteristic	FMT-S	FMT-M	*P* value
Mean age (range)—y	75 (59-91)	74 (49-93)	0.84
Female sex—no. (%)	18 (64%)	21 (75%)	0.56
Median Charlson comorbidity index score[a] (range)	3 (2-9)	3 (1-8)	0.57
First CDI episode refractory to antibiotics—no. (%)	4 (14%)	3 (11%)	1
Recurrent CDI refractory to antibiotics—no. (%)	24 (86%)	25 (89%)	1
Median no. of CDI recurrences (range)	2 (1-5)	2 (1-4)	0.87
Previous pulsed vancomycin therapy before FMT—no. (%)	15 (54%)	11 (39%)	0.42
Previous tapered vancomycin therapy before FMT—no. (%)	4 (14%)	6 (21%)	0.73
Previous fidaxomycin therapy before FMT—no. (%)	9 (32%)	11 (39%)	0.78
Use of systemic antibiotics before CDI—no. (%)	26 (93%)	27 (96%)	1.0
Use of proton pump inhibitors—no. (%)	18 (64%)	21 (75%)	0.56
Hospital-acquired CDI infection—no. (%)	15 (54%)	17 (61%)	0.79
In-patient—no. (%)	28 (100%)	28 (100%)	1.0
Median stool frequency/24 h—no. (range)	6 (4-8)	6 (4-12)	0.54
Pseudomembranous colitis—no. (%)	17 (61%)	19 (68%)	0.78
Leucocyte count—per mm^3			
Median	15490	15570	0.95
IQR	11250-18650	8690-22800	
Leucocyte count >15 000 per mm^3	15 (54%)	14 (50%)	1.0
Median albumin (Range) – g/L	27 (17-36)	27 (18-35)	0.95
Albumin <30 g/dL – no. (%)	16 (57%)	17 (61%)	1.0
Median creatinine (IQR) – mg/dL	1.27 (0.79-2.35)	1.12 (0.66-2.04)	0.17
Median body temperature (IQR) – °C	38.3 (37.7-39)	38.2 (37.9-38.7)	0.58
Body temperature >38.5°C	11 (39%)	10 (36%)	1.0
Severe-complicated CDI—no. (%)	17 (61%)	16 (57%)	1.0

CDI, *C. difficile* infection; FMT, faecal microbiota transplantation; FMT-M, multiple-infusion treatment arm; FMT-S, single-infusion treatment arm; IQR, interquartile range.

[a]Scores on the Charlson co-morbidity index ranges from 0 to 100, with higher scores predicting higher likelihood of hospital mortality (calculated with an online calculator available at https://www.mdapp.co/).[29]

after the first infusion, and were eventually cured. The 19 patients who presented with pseudomembranous colitis at endoscopic evaluation received four faecal infusions on average (range 3-6 infusions).

Overall, FMT-M achieved significantly higher cure rates than FMT-S (100% vs 75%, $P = 0.01$, both in intention-to-treat and per-protocol analysis). None of the cured subjects experienced diarrhoea for causes not related to CDI during the study period.

3.4 | Adverse events

No serious adverse events associated with any of the two treatment protocols were observed.

Eighteen patients in the FMT-S group (64%) and 20 patients in the FMT-M group (71%) experienced transient, self-limiting mild diarrhoea in the first few hours after faecal infusions. Seventeen (61%) patients in the FMT-S group and 23 (82%) patients in the FMT-M group experienced constipation after the treatment, but returned to their usual bowel habit during the follow-up.

4 | DISCUSSION

In this randomised clinical trial, a vancomycin-associated FMT protocol including multiple faecal infusions was significantly more effective than a vancomycin-associated single-infusion FMT protocol in treating severe refractory CDI. Both scheduled treatments were safe, as no serious adverse events occurred during the treatment and observation periods.

This study highlights the efficacy of FMT for the treatment of severe refractory CDI regardless of specific clinical history, as both patients at their first episode of CDI and those experiencing recurrent disease were included. Although this study was not specifically designed to address differences between these two subgroups, our findings suggest that FMT-M may be a promising treatment option for this life-threatening condition also in patients at their first episode of severe or severe-complicated CDI. Further studies, specifically designed to address this issue, are necessary to confirm our data.

In addition, our choice of determining the number of infusions based on the presence of pseudomembranes was appropriate, as this

approach was successful in all 19 patients with pseudomembranous colitis in the FMT-M group, while only 10 of 17 (59%) patients with pseudomembranous colitis in the FMT-S group were cured. This finding confirms both our previous data[8] and those published by Fischer and colleagues.[18,20]

Our study confirms the reliability of colonoscopy as a route of faecal delivery also in a subgroup of frail patients as those with severe disease. Following the protocol used in the FMT-M group, the endoscopic evaluation allows to schedule further faecal infusions based on the presence of pseudomembranes, and therefore; to provide a sustained cure to these patients. In theory, increasing the number of infusions could raise the risk of complications, especially in patients with severely inflamed mucosa. This risk can be minimised by the expertise and skills of the endoscopist, and using caution during the infusion (eg delivering the faecal material in the most proximally reachable section without necessarily getting the caecal fund). By adopting this prudential approach, we did not observe any procedure-related complication in our study.

On the other hand, increased costs associated with repeated procedures were likely offset by the sustained cure of CDI, although we did not perform a cost-effectiveness analysis, as it was not the objective of this randomised trial.

Interestingly, although being less effective than its comparator, the FMT-S protocol was effective in 75% of patients, suggesting that this approach could be attempted with a considerable success rate if physicians are not able to offer multiple faecal infusions.

Another relevant finding is that all failing patients in the FMT-S group experienced a transient clinical improvement before the relapse of *C. difficile*-associated diarrhoea and the worsening of their clinical picture. This finding hypothesises that single-infusion FMT is not sufficient to achieve sustained cure of severe CDI, but could ameliorate temporarily the clinical picture, enabling a response to anti-CDI therapy, as suggested by previous reports.[19]

Moreover, in a recent meta-analysis by Quraishi and colleagues, multiple infusions resulted in a higher CDI cure rate than a single infusion; although in this study, there was no stratification of patients for severity of disease.[25]

The lack of blinding for both participants and investigators constitutes a limitation of this study. Furthermore, steps of the working protocol including a decision-making process (eg the assessment of pseudomembranous colitis) could be operator dependent. However, those with severe refractory CDI represent a frail subgroup of patients, for which blinding could be methodologically desirable but not practical for ethical reasons. In this study, we used indifferently fresh or frozen faeces, as accumulating evidence suggests that both these options are effective in treating CDI[25-28] and this randomised trial was not designed to assess the efficacy of frozen faeces in treating severe CDI. Targeted studies are needed to clarify this issue.

In conclusion, our study is the first randomised clinical trial designed to identify a reliable protocol for severe refractory CDI. We found that a pseudomembrane driven FMT protocol consisting of multiple faecal infusions and concomitant vancomycin was significantly more effective than a single faecal transplant followed by vancomycin in curing this particular clinical picture. As severe CDI is a life-threatening disease with few and poorly effective therapeutic options, this approach could improve the overall health for this patient population, minimise chance of reoccurrence, and decrease the burden of CDI on healthcare. Further studies are needed to confirm our findings, and to extend our results to larger cohorts of patients with severe CDI.

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AUTHORSHIP

Guarantor of the article: Giovanni Cammarota.

Author contributions: Gianluca Ianiro, Giovanni Cammarota, study concept and design; All authors involved in acquisition of data; Gianluca Ianiro, Giovanni Cammarota, analysis and interpretation of data; Gianluca Ianiro, Giovanni Cammarota, drafting of the manuscript; All authors involved in the critical revision of the manuscript for important intellectual content. All authors approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section at the end of the article.

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