

# Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent *Clostridium difficile* infection

G. Cammarota\*, L. Masucci†, G. Ianiro\*, S. Bibbò\*, G. Dinoi\*, G. Costamagna‡, M. Sanguinetti† & A. Gasbarrini\*

\*Institute of Internal Medicine, Catholic University, Faculty of Medicine and Surgery, Rome, Italy.  
†Institute of Microbiology, Catholic University, Faculty of Medicine and Surgery, Rome, Italy.  
‡Institute of Surgery, Catholic University, Faculty of Medicine and Surgery, Rome, Italy.

## Correspondence to:

Prof. G. Cammarota, Department of Internal Medicine, A. Gemelli University Hospital, Largo A. Gemelli 8, 00168 – Roma, Italy.  
E-mail: gcammarota@rm.unicatt.it

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## SUMMARY

### Background

Faecal microbiota transplantation (FMT) from healthy donors is considered an effective treatment against recurrent *Clostridium difficile* infection.

### Aim

To study the effect of FMT via colonoscopy in patients with recurrent *C. difficile* infection compared to the standard vancomycin regimen.

### Methods

In an open-label, randomised clinical trial, we assigned subjects with recurrent *C. difficile* infection to receive: FMT, short regimen of vancomycin (125 mg four times a day for 3 days), followed by one or more infusions of faeces via colonoscopy; or vancomycin, vancomycin 125 mg four times daily for 10 days, followed by 125–500 mg/day every 2–3 days for at least 3 weeks. The latter treatment did not include performing colonoscopy. The primary end point was the resolution of diarrhoea related to *C. difficile* infection 10 weeks after the end of treatments.

### Results

The study was stopped after a 1-year interim analysis. Eighteen of the 20 patients (90%) treated by FMT exhibited resolution of *C. difficile*-associated diarrhoea. In FMT, five of the seven patients with pseudomembranous colitis reported a resolution of diarrhoea. Resolution of *C. difficile* infection occurred in 5 of the 19 (26%) patients in vancomycin ( $P < 0.0001$ ). No significant adverse events were observed in either of the study groups.

### Conclusions

Faecal microbiota transplantation using colonoscopy to infuse faeces was significantly more effective than vancomycin regimen for the treatment of recurrent *C. difficile* infection. The delivery of donor faeces via colonoscopy has the potential to optimise the treatment strategy in patients with pseudomembranous colitis.

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## INTRODUCTION

*Clostridium difficile* is the most common cause of nosocomial diarrhoea related to antibiotics. *Clostridium difficile* is also the aetiological agent of pseudomembranous colitis (PMC), which occurs as result of a toxin-mediated infection. Over the past decade, *C. difficile*-associated diarrhoea has become more frequent, more refractory to antibiotic therapy and more likely to recur.<sup>1–3</sup> The high rate of recurrent disease (which occurs as a consequence of the persistence of spores or re-infection) is a major challenge in treating *C. difficile* infection, with up to 35% of patients experiencing a recurrence after their first *C. difficile* infection episode.<sup>1, 4</sup> Generally, repeated and extended courses of vancomycin are prescribed<sup>5</sup>; however, an effective antibiotic therapy against the recurrences is not yet available. The continued disruption of the normal colonic microbiota by repeated cycles of antibiotics and the persistence of *C. difficile* spores perpetuate the risk of further recurrences (up to 65% after two or more recurrences).<sup>1, 3, 4, 6–15</sup> Recurrences lead to repeated bouts of diarrhoea and greatly expose the patient to the risk of severe complications (i.e. toxic megacolon and septic shock) and the need for surgery.<sup>1, 2, 7, 8</sup> It has not yet been established whether other promising antibiotic-based treatment strategies are effective against recurrent *C. difficile* infection. Fidaxomicin was proven to be more effective than vancomycin in reducing the rate of recurrence, but it has not yet been investigated for the treatment of the recurrences themselves.<sup>16, 17</sup> Moreover, its use in clinical practice is limited by its high cost. Faecal microbiota transplantation (FMT), which consists of the infusion of faecal microbiota from a healthy donor into a recipient individual, has been increasingly utilised as a treatment for patients with recurrent *C. difficile* infection. The process is based on the restoration of the healthy flora in the diseased colon by importing the colonic microbiota of a healthy subject. The route of faecal infusion (i.e. nasogastric or nasojejunal tube, upper endoscopy, retention enema or colonoscopy) often depends on local availability, physician expertise and the route that is deemed safest for the individual patient.

Faecal microbiota transplantation has been performed in hundreds of patients. The outcomes from more than 500 cases have been reported in the medical literature, mostly in noncontrolled case series.<sup>18</sup> Moreover, FMT was demonstrated to be an effective treatment for recurrent *C. difficile* infection in 87% of cases. Colonoscopic delivery showed a slightly higher success rate (93%) than the other routes, such as the placement of a nasoduodenal tube (86%) or even a rectal enema (84%).<sup>18</sup> This

result would seem to indicate that the delivery of the 'healthy' bacteria directly to the entire diseased colonic mucosa may increase the chance of therapeutic success.

To date, only one randomised controlled trial has been performed to evaluate the efficacy and safety of FMT in the treatment of recurrent *C. difficile* infection vs. the standard antibiotic regimen.<sup>19</sup> Using a nasoduodenal tube as the delivery vehicle for the donor microbiota infusion, van Nood et al. achieved a success rate of 81% compared with the 31% and 23% success rates obtained with vancomycin alone or vancomycin with bowel cleaning respectively.

In this study, we conducted a randomised controlled clinical trial with the objective of comparing FMT performed via colonoscopy with a standard vancomycin regimen<sup>20, 21</sup> for the treatment of patients with recurrent *C. difficile* infection.

## MATERIAL AND METHODS

### Study design

In this open-label, randomised controlled clinical trial, we compared the two following treatments: (i) FMT via colonoscopy preceded by bowel cleaning and (ii) the standard vancomycin regimen.<sup>20, 21</sup>

The study was performed at the A. Gemelli University Hospital in Rome. Subjects referred to our hospital were examined by authors involved in the patients' recruitment (G.C., G.I., S.B., G.B. and A.G.) to determine their eligibility for the trial. All enrolled subjects provided their written informed consent. We planned an interim analysis 1 year after the start of the patients' enrolment.

The study protocol was approved by the local ethics committee (A.119/C.E./2013) and registered at ClinicalTrials.gov (NCT02148601). The study was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) Statement.<sup>22</sup>

### Study population

Patients who were at least 18 years of age, had a life expectancy equal to or longer than 3 months and a recurrence of *C. difficile* infection after one or more courses of specific antibiotic therapy (at least 10 days of vancomycin at a dosage of at least 125 mg four times daily or at least 10 days of metronidazole at a dosage of 500 mg three times a day) were considered for inclusion. At enrolment, recurrent *C. difficile* infection was defined as diarrhoea (at least three loose or watery stools per day for 2 or more consecutive days, or at least eight loose

stools in 48 h) and positivity in the *C. difficile* toxin stool test within 10 weeks from the end of the previous antibiotic treatment. To obtain a homogeneous study population in both arms of treatment, only patients we believed able to undergo colonoscopy were enrolled.

The exclusion criteria included prolonged immunodeficiency due to recent chemotherapy; human immunodeficiency virus (HIV) infection; prolonged use of steroids; pregnancy; use of antibiotics other than metronidazole, vancomycin or fidaxomicin at baseline; admission to an intensive care unit; requirement for vasoactive drugs; and other infectious causes of diarrhoea.

**Treatments**

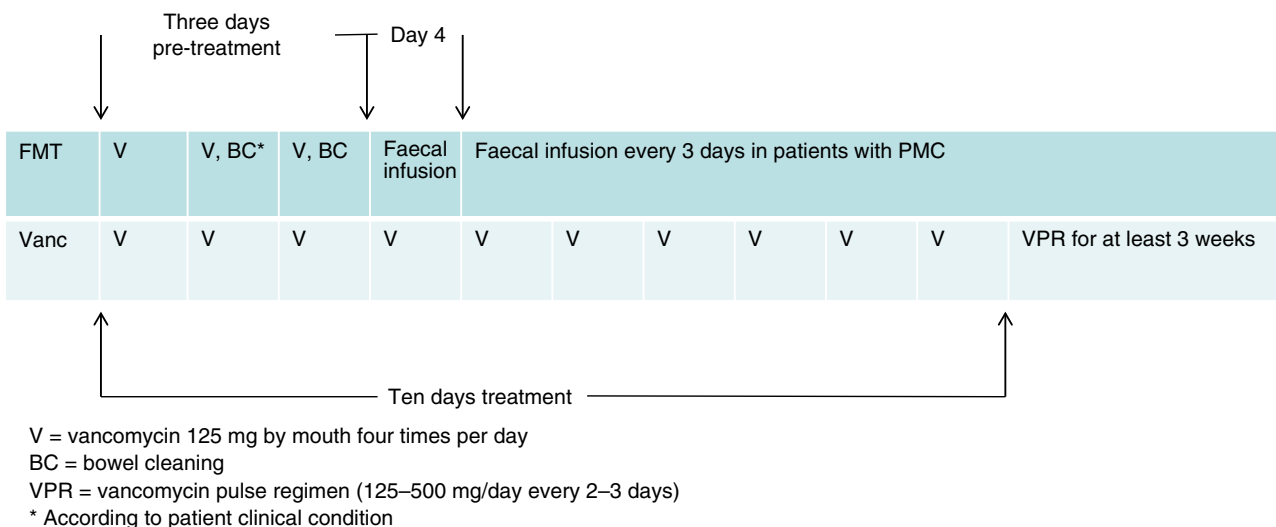
Patients were randomly assigned to one of the following treatments: *FMT* – a short regimen of vancomycin (125 mg by mouth four times a day for 3 days), followed by bowel cleaning with 4 L of macrogol preparation (SELG ESSE) on the last 1 or 2 days (according to the clinical condition of the patients) of antibiotic treatment, followed by consequent faecal infusion from a healthy donor by colonoscopy the next day; or *vancomycin* standard vancomycin treatment of 125 mg by mouth four times daily for 10 days, followed by a pulse regimen (125–500 mg/day every 2–3 days) for at least 3 weeks. Patients in whom recurrent *C. difficile* infection developed after the first faecal infusion were given a second infusion of faeces within 1 week. However, after the enrolment of the first two patients who underwent FMT, this part of the study protocol was amended; thereafter, all subsequent patients with PMC underwent repeated infusions every 3 days

until the resolution of colitis. Patients who had to repeat faecal infusion after 3 days were restricted to a light diet and prepared for colonoscopy by taking only 2 L of bowel preparation before the colonoscopy. Patients in whom the two study treatments failed were re-evaluated to establish whether they were able to receive off-protocol treatment with donor faeces. The timeline of the scheduled treatments is shown in Figure 1.

**Selection and screening of donors**

Healthy volunteers less than 50 years of age (preferably the patient’s relatives or intimates) were initially screened through a specific questionnaire about possible risk factors for potentially transmittable diseases due to their medical history and lifestyle habits. The donors could not have taken antibiotics in the previous 6 months or exhibited significant intestinal symptoms of other intestinal diseases. Other reasons for exclusion of potential donor candidates were: lifestyle associated with increased risk for contracting infections; recent (equal or less than 3 months) travels in tropical areas; new sexual relationship in the last 6 months; recent needle stick accident; previous reception of blood products; body tattoos; gastrointestinal diseases or complaints (abdomen discomfort, alvus disturbances); a family history of gastrointestinal cancer or inflammatory bowel disease; systemic diseases (i.e. diabetes or neurological disorders) or the use of drugs that could be excreted in faeces with potential risk for the recipients.

If no reasons of exclusion from faeces donation were identified through the questionnaire, the candidates



**Figure 1 |** Timeline of scheduled treatments after patient randomisation.

underwent blood and stool exams to exclude potentially transmittable diseases. The blood samples were tested for hepatitis A, B and C, antibodies to HIV-1 and -2, Epstein–Barr virus, *Treponema pallidum*, *Strongyloides stercoralis* and *Entamoeba histolytica*. Blood cell counts and measurements of transaminase, C-reactive protein, albumin and creatinine analysis were also performed. The faeces were tested for *C. difficile* (culture and toxin), enteric bacteria, protozoa and helminths of the large and small bowel, VRE (*vancomycin-resistant Enterococci*), MRSA (*methicillin-resistant Staphylococcus aureus*), and Gram-negative MDR (*multi-drug-resistant*) bacteria.

Before donation, a further questionnaire was used to screen for any recent acute gastrointestinal illnesses, newly contracted infections or other situations that could represent a risk for the patients.

#### Faecal infusion procedure

Faeces were collected by the donor on the day of infusion and rapidly transported to our hospital. In the Hospital's Microbiology Laboratory, the faeces were diluted with 500 mL of sterile saline (0.9%). The deriving solution was blended, and the supernatant strained and poured into a sterile container. Within 6 h after the supply of faeces by the donor, the solution was infused (using 50-mL syringes filled with the solution at the time of colonoscopy) through the operative channel of the scope within the proximal tract of the colon. At the time of infusion, the patients were placed in right lateral recumbency position and were asked to maintain this position for at least 1 h after the procedure to facilitate as much as possible the permanence of the material infused into the proximal portions of the colon. On average, the entire infusion procedure was performed within 10 min, and the tube was removed after the infusion. During the insertion and removal of the colonoscope, the endoscopist was able to make a judgment on the inflammatory conditions of the colonic mucosa and report any additional pathological conditions. Finally, the patients were monitored in the recovery room of the Endoscopy Center for 2 h after the procedures.

#### Outcomes and follow-up

The primary end point was the resolution of diarrhoea associated with *C. difficile* infection 10 weeks after the end of the treatments. For patients in the FMT group who required more than one infusion of faeces, follow-up was extended to 10 weeks after the last infusion. For patients in the vancomycin group, follow-up lasted for 10 weeks after the end of the vancomycin

course. The secondary end point was toxin negative without recurrent *C. difficile* infection 5 and 10 weeks after the end of the treatments.

We defined the cure of *C. difficile* infection as the disappearance of diarrhoea, or persistent diarrhoea explicable by other causes, with two negative stool tests for *C. difficile* toxin. Recurrence after treatment was defined as diarrhoea (at least three loose or watery stools per day for 2 or more consecutive days, or at least eight loose stools in 48 h) unexplainable by other causes, with or without positive stool toxin within 10 weeks from the end of the therapy.

Patients were closely followed up in the days after treatment, and a stool diary was kept by the patients themselves or by family members or the medical and nursing staff. Patients, family members and referral physicians were also questioned about stool frequency and consistency, medication use, and adverse events in the 7 days after the end of treatment and on weeks 2–10 after the end of treatment. Subjects in the vancomycin group were also questioned during the 14-day treatment period. Stool tests for *C. difficile* toxin were performed on weeks 5, 10, and whenever diarrhoea occurred using a Premier Toxins A&B (Liaison *C. difficile* GDH-Toxin A/B – DiaSorin Inc., Stillwater, MN, USA) kit in the central hospital laboratory.

#### Randomisation

Blocked randomisation of subjects was performed by an external person 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.

#### Statistical analysis

Calculation of sample size was based on the superiority of FMT via colonoscopy over the vancomycin regimen. Respectively, a cure rate of 87% for FMT via colonoscopy<sup>18</sup> and 55% for vancomycin<sup>1, 3, 19</sup> were assumed. Considering a two-tailed  $\alpha$  value of 0.05 and a power of 90% ( $\beta = 0.10$ ), the enrolment of 41 patients per group was required. Sample size was calculated with an online software (<http://www.stat.ubc.ca/~rollin/stats/ssize/b2.html>). Considering the possibility of 20% possible dropouts, we planned to enrol 50 patients per group.

Analyses were performed both on an intention-to-treat (ITT) and per protocol (PP) 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 probability test (using two-tailed *P*-values). Because the trial was stopped early after a 1-year interim analysis, the cure rates and odds ratio for the cure rates for the primary end points were calculated with their 99.9% confidence intervals according to the Haybittle–Peto boundary rule (i.e.  $P < 0.001$  for the primary end point). Statistical analyses were performed with an online calculator (<http://www.graphpad.com/quickcalcs/>) and with Microsoft Excel for Mac (Microsoft Excel, Redmond, WA, USA; Microsoft, 2011).

## RESULTS

### Patient recruitment and duration of the trial

From July 2013 through June 2014, 39 patients (F = 23, M = 16, mean age 73 years) were randomly assigned to one of the following treatments: FMT (20 subjects, F = 12, M = 8, mean age: 71 years) or vancomycin regimen (19 subjects, F = 11, M = 8, mean age 75 years). No patient refused the proposed treatment. At the planned 1-year interim analysis, FMT showed a significantly higher efficacy than vancomycin. Therefore, after consulting an independent committee (including two internists and one gastroenterologist), the study was stopped when a total of 39 patients were recruited.

In 23 of the 39 (59%) patients, a positive toxin test before inclusion was confirmed by a positive *C. difficile* culture. All enrolled patients completed the study protocol.

Baseline demographic and clinical characteristics of the patients are provided in Table 1.

### Donors

Most approved donors were relatives of the patients (16 subjects); in two cases, the donors were intimates and in another two FMT cases, the donor was unrelated because the patients had no relatives. The unrelated donor was a subject that had previously served as a donor for his relative. Therefore, faeces from a total of 18 donors were used to treat 20 subjects in the infusion group. A mean ( $\pm$ s.d.) of  $152 \pm 32$  g of faeces was infused. The mean time from defecation to infusion was  $3.8 \pm 0.8$  h.

### Study outcomes

The first two patients in the FMT group had PMC. One of these two subjects had a recurrence after 1 week after an initial resolution of symptoms and thus underwent another faecal infusion. Although with the second procedure, we noted a partial reduction in the presence of pseudo-membranes and initial symptom resolution, the patient had another recurrence after 5 days. Therefore, the patient was treated with vancomycin but died from sepsis after 1 week. The second patient with PMC was affected by severe cardiopathy; he was initially treated with a faecal infusion. After an apparent initial amelioration of diarrhoea with an objective reduction in the thickening of the colonic wall during the TC examination, the

**Table 1** | Baseline demographic and clinical characteristics of the patients

Characteristic	FMT	Vancomycin	<i>P</i> -value
Mean age (range), year	71 (29–89)	75 (49–93)	0.4568
Female sex, <i>n</i> (%)	12 (60)	11 (58)	1.0000
Median Charlson Comorbidity Index (range), score*	2 (0–5)	2 (1–5)	0.3498
Median recurrences of <i>Clostridium difficile</i> infection (range), <i>n</i>	3 (2–5)	3 (1–4)	0.3545
Previous tapered vancomycin therapy, <i>n</i> (%)	19 (95)	16 (84)	0.3416
Antibiotic use before CDI, <i>n</i> (%)	20 (100)	19 (100)	1.0000
Hospital-acquired CDI infection, <i>n</i> (%)	10 (50)	14 (74)	0.1908
Admitted to a hospital at study inclusion, <i>n</i> (%)	15 (75)	16 (84)	0.1606
Use of proton pump inhibitor, <i>n</i> (%)	11 (55)	13 (68)	0.5145
Median stool frequency per 24 h (range), <i>n</i>	6 (2–15)	6 (2–12)	0.5539
Leucocyte count – per mm <sup>3</sup>			
Median	10 100	9900	0.8835
Range	3500–22 000	5000–19 100	
Albumin (g/dL)	3.6 (2.9–4.4)	3.4 (2.9–4.4)	0.1605
Median creatinine (range), mg/dL	1.2 (0.7–2.5)	1.2 (0.8–1.7)	0.7586

\* Scores on the Charlson comorbidity index ranges from 0 to 100, with higher scores indicating improved functional status.

patient had a recurrence 5 days after the faecal infusion procedure. Due to the deterioration of the general clinical condition of this patient, we could not offer him a second infusion of faeces. Therefore, the patient started vancomycin treatment. The patient died from sepsis and pulmonary oedema 15 days later. Based on the ITT and PP analysis, we considered these two patients as FMT failures. Therefore, after these first two experiences with FMT, we decided to amend the FMT protocol to treat subjects with PMC with repeated faecal infusions every 3 days until the resolution of colitis was achieved.

**FMT group.** In the FMT group, 13 of the 20 patients (65%) were cured after the first infusion of healthy donor faeces; none of these 13 subjects were diagnosed with PMC. The seven remaining patients were diagnosed with PMC; six of these patients received multiple infusions (four patients received two infusions; one patient received three infusions; and one underwent four infusions), and one patient received only one infusion. Overall, five of the seven patients with PMC were cured, while two had experienced a recurrence. These two subjects who received one or two infusions (the latter with an interval of 1 week) subsequently died from apparent *C. difficile*-related clinical complications. All five patients with PMC who were cured received a faecal infusion procedure every 3 days until the resolution of colitis was achieved. In these patients, we were able to observe the progressive disappearance of pseudo-membranes with the endoscope.

Overall, donor faeces cured 18 of the 20 patients (90%). At the time of writing this paper (October 2014), all cured FMT patients are still living with the exception of one patient who died 8 months after discharge following a heart attack.

**Vancomycin group.** Five of the 19 patients (26%) were cured. Two patients were refractory to the antibiotic treatment and died from *C. difficile*-related complications, whereas the remaining 12 patients had a *C. difficile* recurrence. The median time to recurrence was 10 days (range, 4–21 days) after the end of the vancomycin treatment. At the time of recurrence, the latter patients had been previously discharged home from the hospital after an initial resolution of symptoms, and therefore we were not able to offer them the faecal infusion. At the time of writing this paper, we contacted all of these patients by phone to determine their clinical conditions, and found that they underwent repeated cycles of antibiotic therapy (including vancomycin and metronidazole) against further recurrences of *C. difficile* infection after the end of the study: seven

patients had resolution of symptoms (five of them with documented negative toxin test results) after 1–3 cycles of therapy (however, two of these subjects died 6 and 8 months after hospital discharge from severe heart failure and from prostate cancer-related complications, respectively); two patients died from unspecified causes 3 and 5 months after discharge without a clear resolution of *C. difficile* infection symptoms and without providing data on their *C. difficile* status; three patients were lost during follow-up.

**Overall outcome analysis.** Overall, FMT by colonoscopy achieved significantly higher remission rates of recurrent *C. difficile* infection compared to vancomycin treatment (90% vs. 26%,  $P < 0.0001$  for both ITT and PP analysis) (Figure 2). For FMT vs. vancomycin treatment, the overall odds ratio of the cure rates was 25.2 [99.9% confidence interval (CI) from 1.26 to 502.30]. At 5 and 10 weeks from the end of the treatments, 18 of the 20 patients in the infusion group had negative *C. difficile* stool toxins; the remaining two patients had a positive stool toxin within 1 week after the treatment. In the vancomycin group, three subjects were negative for stool toxin after 5 weeks, and five patients were negative after 10 weeks.

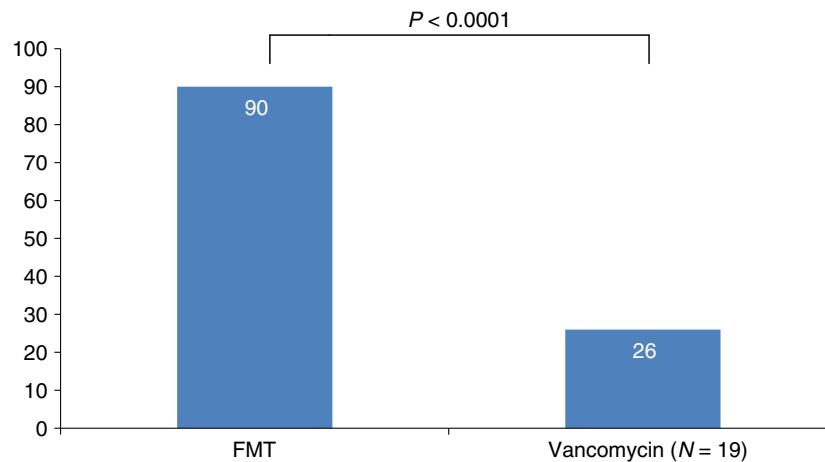
None of the cured patients had episodes of diarrhoea for causes not related to *C. difficile* infection during the study period.

#### Adverse events

Immediately after donor faeces infusion, 19 of the 20 (94%) patients had diarrhoea, and 12 of the 29 patients experienced (60%) bloating and abdominal cramping. In all patients, these symptoms resolved within 12 h. During follow-up, all patients who were treated with donor faeces returned to their alvus habits. No adverse events specifically relatable to the vancomycin regimen were reported.

## DISCUSSION

In this small, open-label randomised controlled trial, we found that FMT via colonoscopy preceded by a 3-day vancomycin regimen was more effective compared to a standard 2-week vancomycin scheduled treatment against recurrent *C. difficile* infection. It is worth noting that the 3-day vancomycin regimen prior to FMT was offered to patients to avoid leaving them uncovered by any medical treatment for the time required for donor screening. FMT achieved a 90% eradication rate in the absence of any significant adverse events. Interestingly, no patient refused the proposed treatment or expressed concerns about any aspect of FMT. The findings of this study are consistent with those reported in the literature, and in particular



**Figure 2** | Percentage of patients cured.

confirm and extend beyond the previous excellent success rate (81%) obtained in a randomised controlled trial using a nasoduodenal tube for faecal infusion.<sup>19</sup> We also confirm the extremely low response rate of a vancomycin regimen in curing these subjects (26% vs. the 31% reported by van Nood *et al.*).<sup>19</sup> Before their inclusion, most patients had relapses after previous vancomycin-based treatments; this most likely contributed to the poor results of the vancomycin regimen in our study.

A limitation of this study is the lack of blinding for either the participants or the investigator. However, recurrent *C. difficile* infection represents a difficult clinical challenge, for which blinding may be desirable but not practicable.

Our trial was designed to evaluate faecal infusion via colonoscopy to treat recurrent *C. difficile* infection because from our own perspective, this route of administration is potentially able to offer several advantages. First, colonoscopy allows the physicians to evaluate the severity of inflammation and to detect the presence or absence of PMC, a condition which is associated with more severe *C. difficile* infection. Although there are multiple laboratory and clinical markers of disease severity (i.e. white blood cell count, albumin level, fever and evidence of end-organ damage),<sup>23, 24</sup> standardised scores able to predict PMC are lacking, and this peculiar condition may only be truly diagnosable by colonoscopy.

Importantly, we found that PMC was present in 35% of patients (7 of the 20 patients undergoing colonoscopy) with recurrent *C. difficile* infection, confirming the result of a previous report.<sup>25</sup> PMC patients seemed to require multiple faecal infusions to be cured. In our study, after the failures of two faecal infusions in the first subjects

with PMC, we decided to adopt an every 3-day faecal infusion strategy until the resolution of colitis was achieved. Following this regimen, the subsequent five patients with this severe form of colitis were all cured. In these patients, we were able to observe with the endoscopic the spectacular progressive disappearance of pseudo-membranes until their resolution. We are aware that the increase in the number of faecal infusion procedures increases both the risk of complications and cost. The latter is related to the repeated colonoscopies because we used the same healthy donor for each subject. However, in this study, we did not observe an increase in complications in subjects who underwent multiple infusions of faeces, and the cost increase was more than offset by halting *C. difficile* infection recurrences.

Another advantage of FMT performed via colonoscopy is the possibility of infusing large volumes of suspensions directly into the site of inflammation in the proximal segments of the colon. The latter objectively favours a better retention of the infused material compared with enemas. Undoubtedly, colonoscopy carries a small risk of perforation, and this possibility is likely enhanced in patients with severely inflamed mucosa. However, this risk can be minimised by the experience and skill of the endoscopist. In our study, all FMT patients completed the colonoscopic examination, and in all patients, the infusion was introduced into the caecum/ascending colon. Moreover, the procedure-related risk of complication of colonoscopy is theoretically balanced by the hypothetical risks associated with the use of the nasogastric/duodenal tube (i.e. perforation of the upper gastrointestinal tract, aspiration and vomiting) apart from the necessity of using a radiological control for tube placement.

A recent trial including a very small cohort of patients attempted to compare the two routes of faecal infusion (colonoscopy vs. nasogastric tube) showed a slight superiority for the colonoscopy route (80% vs. 60% respectively); however, the difference between the two treatment approaches was not significant.<sup>26</sup> In addition, this study did not include a non-FMT control group.

Finally, we would discuss one last consideration from the analysis of our data. After we optimised the protocol on the basis of the endoscopic view, 18 of the 18 (100%) subsequent patients who were treated with faecal infusion were cured. These data seem to suggest that the number of infusions can vary depending on the severity of the inflammation of the colonic mucosa and that the endoscopic picture could play a role in deciding the FMT strategy in a given patient. However, because head to head comparisons of various FMT protocols, including differences in the number and routes of infusions, have never been performed, conclusions cannot be drawn in this regard and our results have to be interpreted with caution. An additional limitation of this study is that vancomycin patients did not undergo colonoscopy and we do not know how many vancomycin subjects had PMC. This limitation is due to the intrinsic difficulty in recruiting vancomycin subjects for colonoscopy. However, as the baseline demographic and clinical characteristics of patients enrolled were similar in the two study groups (Table 1), we can only suppose that a similar number of PMC cases were present (and not detected) in the vancomycin group. On the other hand, biomarkers which could in theory act as indicators of disease, disease recurrence and disease stratification and therefore help to effectively direct *C. difficile* infection therapies (including FMT) are lacking. Some studies have suggested that faecal calprotectin and faecal lactoferrin have limited applicability, although these biomarkers have not yet been fully investigated in this role.<sup>27</sup> In another study, severe white blood cell count elevation and a rise in creatinine to >50% above baseline were demonstrated as independent predictors of serious adverse events due to *C. difficile* infection; however, these markers were not fully investigated as predictors of disease recurrence.<sup>28</sup> In contrast, Shivashankar *et al.* demonstrated that increasing age and antibiotic use were associated with recurrent *C. difficile* infection, whereas peripheral leucocyte counts and changes in serum creatinine levels more than 1.5-fold were not.<sup>29</sup>

Interestingly, a recent pilot study experimented with an innovative modality for the administration of faecal material as frozen FMT capsules from previously screened unrelated donors in patients with recurrent *C. difficile* infection. Resolution of diarrhoea was obtained in 70% of patients after a single capsule-based FMT and in an overall 90% of patients after the re-treatment of failures without adverse events.<sup>30</sup> The main limitations of this study were the small sample size and the lack of placebo or active comparisons. Therefore, further studies are needed to explore the real potentiality of this type of faecal administration. In addition, other studies will be necessary to confirm the possibility of the preparation of frozen transplants from healthy universal donors to simplify the practical aspects of FMT without a loss of efficacy or safety.<sup>31</sup>

In conclusion, the infusion of donor faeces resulted in better treatment outcomes compared to vancomycin therapy in patients with recurrent *C. difficile* infection. Although further studies are needed to confirm our findings, the route of administration via colonoscopy appears to have the potential to optimise the treatment according to the severity of inflammation of the colonic mucosa at the time of endoscopy.

## AUTHORSHIP

*Guarantor of the article:* None.

*Author contributions:* Giovanni Cammarota was involved in the study concept and design; in recruiting and treating patients; in the analysis and interpretation of data; in drafting the manuscript; in the critical revision of the manuscript; in obtaining funding for the study. Luca Masucci and Maurizio Sanguinetti were involved in the study concept and design and in the microbiological analysis. Gianluca Ianiro and Stefano Bibbò were involved in recruiting patients and in the acquisition and analysis of data. Giorgia Dinoi was involved in recruiting patients. Guido Costamagna was involved in the critical revision of the manuscript. Antonio Gasbarrini was involved in recruiting patients and in the critical revision of the manuscript. All authors approved the final version of the manuscript.

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