Early Fecal Microbiota Transplantation Improves Survival in Severe Clostridium difficile Infections

Marie Hocquart,1,2 Jean-Christophe Lagier,1 Nadim Cassir,1 Nadia Saidani,1 Carole Eldin,1 Jad Kerbaj,1 Marion Delord,1 Camille Valles,1 Philippe Brouqui,1 Didier Raoult,1 and Matthieu Million1

1Aix Marseille Université, CNRS, IRD, INSERM, AP-HM, URMITE, IHU Méditerranée Infection, and 2Département Universitaire de Médecine Générale, Aix Marseille Université, AP-HM, Marseille, France

(See the Editorial Commentary by Andremont on pages 651–2.)

Background. Severe Clostridium difficile infections (CDIs) are associated with a high mortality rate despite medical and/or surgical treatment. Fecal microbiota transplantation (FMT) prevents recurrences, but its effect on survival has been shown only in patients with O27 ribotype CDI. Here, we investigated whether early FMT could improve survival in hospitalized CDI patients, particularly those with severe infection.

Methods. We performed a retrospective cohort study between May 2013 and April 2016 at the infectious diseases department of the North University Hospital of Marseille, France. Patients received either medical treatment alone or treatment with early FMT. The primary outcome was the 3-month mortality rate.

Results. A total of 111 patients were included: 66 in the FMT group and 45 in the non-FMT group. No patient underwent surgery. The O27 ribotype (odds ratio [OR], 3.64 [95% confidence interval [CI], 1.05–12.6], P = .04), severe CDI (OR, 9.62 [95% CI, 2.16–42.8], P = .003), and FMT (OR, 0.13 [95% CI, .04–.44], P = .001) were independent predictors of 3-month mortality. FMT improved survival in severe cases (OR, 0.08 [95% CI, .016–.34], P = .001) but not in nonsevere cases (OR, 1.07 [95% CI, .02–56.3], P = .97), independent of age, sex, comorbidities (Charlson score), and ribotype. The number of severe patients who needed to be treated to save 1 life at 3 months was 2.

Conclusions. Early FMT dramatically reduces mortality and should be proposed as a first-line treatment for severe CDI. Further studies are needed to clarify complications and contraindications. Surgery should be reassessed in this context.

Keywords. Clostridium difficile; fecal microbiota transplantation; mortality; survival; treatment.

Clostridium difficile infection (CDI) is an emerging worldwide public health concern associated with substantial mortality and recurrence [1–3]. The mortality rate dramatically increased from 1.5% before 2000 to 4%–6% and 7%–17% in recent endemic and epidemic periods, respectively, in the United States [2]. The mortality rate for severe infection is much higher, reaching 36%–58% without surgery and 32%–57% with surgery [4–7], with no significant difference in the largest meta-analysis to date [8]. Moreover, only 30% of patients with severe CDI underwent surgery, in relation to a selection bias [8]. The emerging epidemic hypervirulent O27 ribotype has been associated with high mortality [9] (64% in our unit in 2013–2014 [10]). The binary toxin is also considered as a virulence factor associated with CDI severity [11]. Other predictive factors of mortality include age, usual comorbidities, severity, and specifically, inflammatory bowel disease [12, 13]. Among antimicrobial agents to treat CDI, vancomycin was shown to be superior to metronidazole, especially for severe colitis [4, 14], but resistance is increasing [15]. Fidaxomicin is not superior to vancomycin [16], while rifaximin [17], tolevarmin [18] and nitazoxanide [19] have been successful as salvage therapy. Other nonmicrobial treatments have no demonstrated efficacy [20–22].

Bacteriotherapy, predominantly fecal microbiota transplantation (FMT), has revolutionized the management of patients with CDI. The efficacy of FMT on recurrence has been reported to range from 70% to 100% in several studies in the literature (Supplementary Table 1). The dramatic efficacy of FMT vs vancomycin was demonstrated on the absence of relapse [23, 24]. We first showed that early FMT improved survival in patients with CDI caused by the hypervirulent O27 ribotype [10]. In severe CDI, the efficacy of FMT in preventing recurrences and decreasing the duration of symptoms has been demonstrated, though not the efficacy against mortality [25, 26]. Subsequently, we successfully treated 2 patients with non-O27 ribotype CDI but with severe colitis, including 1 patient in the intensive care unit [27]. Based on the continuing ongoing epidemic, the demonstrated efficacy of FMT in the CDI context, the demonstrated biological mechanism (disruption of the gut microbiota during CDI restored by FMT) [28, 29], and the relatively good safety and long-term tolerance [30–32], we subsequently proposed early
FMT to all CDI patients hospitalized in our unit regardless of comorbidities, ribotype, recurrence, or severity. Assessments of early FMT efficacy are lacking in this context and are of foremost importance. Moreover, although the efficacy against recurrence has been extensively studied [23, 24] (Supplementary Table 1), comparative studies evaluating the role of FMT in improving mortality are lacking, especially in non-O27 and none severe CDI.

The objective of this study was to compare the mortality of all patients hospitalized for CDI in our unit with and without FMT. Moreover, as the FMT method changed during the period of inclusion (fresh to frozen stool [33]), we compared the efficacy of these 2 methods on mortality.

**METHODS**

**Study Population and Center**

We performed a 3-year monocentric, retrospective cohort study with prospective collection of data following the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines [34] (Supplementary Data 1). The study population was all patients hospitalized for colitis with *C. difficile* in the infectious diseases department of the North University Hospital of Marseille from May 2013 to April 2016. Patient information was extracted from the hospital computerized database DIM (département d’information médicale) using the A047 code “pseudomembranous colitis” with colitis and/or *C. difficile* in primary or secondary diagnosis, and data were collected using a standardized questionnaire, including age, sex, comorbidities (Charlson score [35]), *Clostridium difficile* episodes, and interventions (Supplementary Data 2). Patients were followed up at 3, 6, and 12 months. A phone call was used to obtain information for those not presenting at control visits. The exclusion criteria were lack of patient consent, age <18 years, and missing data not allowing us to confirm age, sex, diagnosis, and treatment. The study was approved by the local (Institut Hospitalo-Universitaire Méditerranée Infection) ethics committee, number 2017-009. Each patient provided oral or written informed consent.

**Definitions**

CDI was defined as previously reported [13] by the association of diarrhea, ileus, or endoscopic colitis associated with microbiological evidence (positive polymerase chain reaction with systematic research of the O27 ribotype and the binary toxin). Severe colitis was defined by leukocytes >15 g/L, albumin <30 g/L, serum creatinine >130 μmol/L or >1.5 times the baseline, peritonitis, occlusive syndrome, megacolon, or signs of shock.

**Treatments**

The fecal infusion preparation and procedure were previously reported [10], except that fresh stools were used from May 2013 to April 2015, whereas frozen stools (–80°C) were used from May 2015 to April 2016. Donors (<65 years of age) were relatives or anonymous volunteers selected by a questionnaire and microbiological analyses (blood and feces) according to the 2015 French recommendations of the National Agency for the Safety of Medicines (ANSM) [36] (Supplementary Data 3 and 4). FMT was routinely available with a prespecified protocol [10], but the decision to transplant was left to the discretion of the clinician in charge of the unit at that time (N. S., C. E., J. K., M. D., J. C. L., M. M., or P. B.). Agents and dosages of antimicrobial treatment were as follows: for the FMT group: vancomycin 500 mg 4 times daily 2 days before and 4 days after FMT for patients with the O27 strain or severe disease (urgent FMT protocol), 4 days before and 4 days after for non-O27 strains (standard FMT protocol); and the no-FMT group: vancomycin orally (500 mg 4 times daily for 10 days), metronidazole orally or intravenously (500 mg 3 times daily for 10 days), vancomycin associated with metronidazole (same dosages), or fidaxomicin orally (200 mg 3 times daily for 10 days) [13], according to recurrence, severity, and sensitivity to treatments.

**Outcomes**

The major outcome was the 3-month all-cause mortality rate. As previously reported [37], CDI was deemed the attributable cause of death if we judged that the patient would not have died within 3 months in the absence of CDI. Death was considered to be directly related, indirectly related, or unrelated to CDI (Supplementary Table 2). The secondary objective was to evaluate the tolerance for the various treatments and to compare fresh or frozen FMT, O27 or non-O27 ribotype, and severe or none severe colitis.

**Potential Predictors and Confounding Factors**

In addition to FMT, age, sex, Charlson score, O27 ribotype, binary toxin, hospital/retirement home–acquired CDI, number of previous episodes, severe colitis, and mode of FMT preparation (fresh or frozen) were tested as independent variables in different models.

**Statistical Analysis**

We compared the 3-month survival rates of patients with or without FMT. Only the first 3 months were considered because most (>50%) deaths occurring after 3 months were unrelated to CDI (Supplementary Table 2). Student t test or Mann-Whitney test, when appropriate, was used to perform 2-group comparisons for quantitative variables. The χ² test or Fisher exact test, when appropriate, was used for qualitative variables. Variables with P < .20 in the univariate analysis or considered to be clinically important were entered into multiple logistic regression models to identify independent predictors of 3-month mortality. Preplanned subgroup analyses included type of preparation (fresh vs frozen), ribotype (O27 vs non-O27), severity (severe vs none severe) and patients treated with vancomycin. To test the robustness of our results, including patients lost to follow-up before 3 months, we used univariate (Kaplan-Meier curve with log-rank test) and
RESULTS

Study Population

One hundred thirty patients were screened, and 19 were excluded: 14 were ultimately not hospitalized in our department, 1 had colitis without *C. difficile*, and 4 had missing data. Overall, 111 patients were included: 66 had FMT (51 had 1 transplant, 14 had 2 transplants, and 1 had 3 transplants in cases of relapse with good tolerance of the first transplant), 45 patients received medical treatment alone and no transplant because 4 patients refused, 1 was no longer symptomatic in our service, 1 had an unexpected immunodeficiency and was considered contraindicated, and 39 were ultimately rejected according to the physician’s discretion (26 were considered to have had good progression with antibiotics alone, and 13 were considered too unstable to undergo the transplant; these assessments were purely subjective and physician dependent) (Figure 1). Antimicrobial agents included vancomycin (101 patients), metronidazole (26 patients, all in the non-FMT group), and fidaxomicin (4 patients, all in the no-FMT group). One patient did not receive antimicrobial agents in our unit because of spontaneous resolution of diarrhea. Dosages followed the local protocols (see Methods).

Our population was older than many published studies (median age, 82 years [interquartile range, 72–88 years]; range, 34–101 years). There were no significant differences in baseline characteristics between the 2 groups (Table 1) except for the number of recurrences before hospitalization in our unit, which was significantly increased in the FMT group (P = .02). This finding was expected considering the current recommendations.

![Study flowchart](Image)

**Figure 1.** Study flowchart.

### Table 1. Baseline Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>FMT (n = 66)</th>
<th>No FMT (n = 45)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, median (IQR)</td>
<td>81 (69–87)</td>
<td>83 (72–88)</td>
<td>.84‡</td>
</tr>
<tr>
<td>Age &gt;65 y</td>
<td>60 (91)</td>
<td>36 (80)</td>
<td>.10§</td>
</tr>
<tr>
<td>Age &gt;80 y</td>
<td>36 (54)</td>
<td>26 (58)</td>
<td>.74¶</td>
</tr>
<tr>
<td>Male sex</td>
<td>23 (35)</td>
<td>13 (29)</td>
<td>.51※</td>
</tr>
<tr>
<td>Charlson comorbidity index, median (range)¶</td>
<td>2 (1–3)</td>
<td>2 (1–4)</td>
<td>.17#</td>
</tr>
<tr>
<td>WHO performance status, median (range)##</td>
<td>3 (1–3)</td>
<td>2 (2–3)</td>
<td>.20*</td>
</tr>
<tr>
<td>Patients with dementia</td>
<td>15 (23)</td>
<td>12 (27)</td>
<td>.63#</td>
</tr>
<tr>
<td>Bedridden patients</td>
<td>27 (41)</td>
<td>18 (40)</td>
<td>.92#</td>
</tr>
<tr>
<td>Use of proton pump inhibitor</td>
<td>30 (45)</td>
<td>22 (49)</td>
<td>.72#</td>
</tr>
<tr>
<td>Recent admission to a hospital*</td>
<td>54 (82)</td>
<td>35 (78)</td>
<td>.60#</td>
</tr>
<tr>
<td>Antibiotic use before CDI†</td>
<td>57 (87)</td>
<td>38 (84)</td>
<td>.78#</td>
</tr>
<tr>
<td>Hospital/retirement home-acquired CDI</td>
<td>44 (67)</td>
<td>28 (62)</td>
<td>.63#</td>
</tr>
</tbody>
</table>

Binary toxin: 34 (51) (27 (60) .77. Ribotype O27: 28 (42) (25 (56) .17. Median recurrences, No. (range): 0 (0–5) (0 (0–3) .02. Severe colitis: 37 (56) (27 (60) .68. Leukocyte count, median (IQR): 11.1 (7.4–18.4) (13.7 (7.6–18.7) .59. Leukocyte count >15 000 cells/mL: 25 (38) (18 (40) .82. Albumin, g/L, median (IQR): 31.3 (25.7–35.2) (31.6 (24.6–34.7) .49. Albumin <30 g/L: 31 (47) (20 (44) .79. Serum creatinine, median (IQR): 91.8 (64.0–161.5) (79.9 (69.2–132.6) .22. Serum creatinine >133 µmol/L or >1.5 times the premorbid level: 16 (24) (13 (29) .58.

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation; IQR, interquartile range; WHO, World Health Organization.

*Two-sided Mann-Whitney test.

†Two-sided χ² test.

‡Charlson score: index of comorbidities corresponding to the 1-year mortality risk.

§WHO performance status: patient autonomy indicator from 0 to 4.

More than 2 days in the previous 3 months.

In the 3 months preceding.

Study Outcomes

The global mortality rate was 24.3% (27/111) at 3 months after the diagnosis of CDI: 12.1% (8/66) in the transplant group vs 42.2% (19/45) in the antibiotic group (univariate odds ratio [OR], 0.19 [95% confidence interval [CI], .073–.49], P < .0003). No patient underwent colectomy. Antimicrobial agents could not be compared, as all patients in the FMT group received vancomycin and none received either metronidazole or fidaxomicin. Consequently, vancomycin and FMT were perfect predictors and could not be included together in our models. In the FMT group, there were 6 (2 direct and 4 indirect) deaths related to CDI and 2 deaths unrelated to CDI. In the no-FMT group, there were 15 (12 direct and 3 indirect) deaths related to CDI and 4 unrelated deaths (Supplementary Table 2). There was only 1 death among patients <65 years of age (0/66 in the FMT group and 1/45 [2.2%] in the no-FMT group). Most of the deaths occurred in patients between 81 and 90 years of age (Supplementary Figure 1). Six (5.4%) patients were lost to multivariate (Cox model regression) survival analysis. Finally, we determined the number needed to treat to save a life (prevent a death) at 3 months. A P value <.05 was considered statistically significant in all comparisons. The analyses were performed using SPSS software version 20.0 (IBM, Paris, France).
follow-up before 3 months (5 in the FMT group [25, 35, 49, 52, and 67 days] and 1 in the no-FMT group [29 days]).

Hospital/retirement home-acquired CDI, the number of previous episodes, and the binary toxin were not associated with 3-month mortality in any of these models (Supplementary Tables 3–5). Accordingly, all final multivariable models included age, sex, Charlson score, O27 ribotype, severe colitis, and FMT. Age (OR, 1.07 [95% CI, 1.002–1.14], P = .045), Charlson index (OR, 1.46 [95% CI, 1.07–1.99], P = .02), the O27 ribotype (OR, 3.64 [95% CI, 1.05–12.6], P = .04), severe CDI (OR, 9.6 [2.2–42.8], P = .003), and FMT (OR, 0.13 [95% CI, .04–.44], P = .001) were independent predictors of 3-month mortality. The number of patients who needed to be treated to save 1 life at 3 months was 4 (95% CI, 2.2–7.5).

Subgroup Analyses
Thirty (45.5%) transplants were performed using fresh stools and 36 (54.5%) with frozen stools. Among patients with FMT, no significant difference was found between frozen and fresh FMT (logistic regression: OR, 2.8 [95% CI, .44–18.0], P = .28). FMT decreased mortality regardless of the O27 ribotype (O27 positive: OR, 0.14 [95% CI, .03–.61], P = .009; O27 negative: OR, 0.072 [95% CI, .005–1.10], P = .059). In contrast, FMT decreased mortality only in patients with severe colitis. A total of 6 of 34 (17%) patients died in the FMT group vs 18 of 26 (69%) in the non-FMT group (bilateral χ² test, P < .0001). In this group, the number of patients who needed to be treated to save 1 life at 3 months was 2 (95% CI, 1.4–3.4). This finding was confirmed in the multivariate analyses (severe CDI: OR, 0.075 [95% CI, .016–.34], P = .001; nonsevere CDI: OR, 1.07 [95% CI, .02–56.3], P = .97).

Because vancomycin could have been responsible for the observed improved prognosis because it was administered in 66 of 66 (100%) patients in the FMT group according to local protocols (see Methods) but in only 35 of 45 (78%) in the no-FMT group (Fisher exact test, P < .0001), we performed a subgroup analysis of the 101 patients treated with vancomycin (only 10 included patients did not receive vancomycin). FMT remained associated with a similar improved survival (OR, 0.08 [95% CI, .02–.32], P = .003), confirming that the choice of the antimicrobial agent was not responsible for the observed effect.

Survival Analysis
To test the robustness of our results, including the 6 patients lost to follow-up before 3 months, we performed a survival analysis; FMT was associated with a very significant decrease in mortality (log-rank test, P < .0002; Supplementary Figure 2), which was confirmed in a Cox regression model (hazard ratio [HR], 0.23 [95% CI, .095–.541], P = .001; Supplementary Figure 3). FMT reduced mortality only in patients with severe CDI (log-rank test, P < .0001; Figure 2A) but not those with nonsevere CDI (P < .86; Figure 2B). This finding was confirmed in the multivariate analyses (severe CDI: HR, 0.15 [95% CI, .06–.39], P < .0001; nonsevere CDI: HR, 0.61 [95% CI, .02–20.5], P = .78), consistent with the logistic regression analyses.

DISCUSSION
Here, we showed that early FMT dramatically prevented mortality in patients with severe CDI. Risk of bias was low because baseline characteristics were similar and multivariate survival models were used. Antimicrobial agents are unlikely to play a confounding role, as effect size was unchanged, selecting only

Figure 2. Effects of fecal microbiota transplantation (FMT) on Clostridium difficile infection (CDI) survival according to severity. Green line: FMT. Blue line: No FMT. A, Early FMT dramatically reduced mortality in patients with severe CDI (Kaplan-Meier curve, log-rank test, P < .0001). B, Early FMT did not reduce mortality in patients with nonsevere CDI (Kaplan-Meier curve, log-rank test, P = .86).
patients treated with vancomycin. In our study, FMT by nasogastric tube was well tolerated, with no serious adverse effects. In a recent systematic review [30], the death rate attributed to FMT was evaluated to be 3 of 1190 (0.25%), which seems very low compared with CDI-attributable mortality in patients with severe infection without FMT (69% in this study). Adverse events mainly included mild gastrointestinal symptoms and fever. Severe complications included infections, autoimmune complications, and exacerbation of inflammatory bowel disease.

According to some authors, double-blind, randomized, multicenter trials should be performed in different countries before modifying the recommendations for first-line treatment of patients with severe CDI. However, clinical judgment should always guide methodology and statistical analysis [38]. In fatal emerging infections, such as human immunodeficiency virus in 1990 [39], Ebola in 2015 [40], and currently C. difficile, the use of nonrandomized trials was justified only when 5 conditions were met: (1) patients who do not receive treatment have a uniformly unfavorable prognosis; (2) the treatment should not be associated with serious and substantial side effects; (3) the potential benefit is sufficiently high to merit a trial; (4) the scientific rationale must be strong enough for a positive result to be generally accepted; and (5) there should be no other appropriate treatment for use as a control. Moreover, according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group [41], beyond the quality of evidence, the judgment on the strength of a recommendation requires considering the following elements in addition to those already mentioned: (6) “directness,” which is the fact that the population, intervention, and measurement of the outcome in the study are similar to those of interest under real circumstances; (7) the relative importance of the outcome (critical or noncritical, surrogate); (8) the risk-benefit ratio; and (9) the cost (use of resources).

Regarding our study, (1) patients with severe CDI without FMT had a very severe prognosis with very high mortality (30%–60%); (2) FMT has a good safety profile compared with CDI and surgery; (3) the potential benefit was sufficiently high (OR, <0.2 [41]) to be unambiguous; (4) the scientific rationale, including all available clinical studies taking recurrence as outcome and basic research studies on microbiota restoration, was sufficiently strong for the result to be generally accepted; (5) surgery, usually proposed as salvage therapy in severe cases, was not an appropriate treatment to serve as a control because it could be proposed only in selected patients (30%), the benefit was not demonstrated, mortality remained very high in operated patients (32%–57%) [4–8], and the quality of life in survivors is strongly altered; (6) directness seemed optimal, as our study, conducted in the epicenter and period of a C. difficile epidemic [9], included a population that would benefit the most from first-line FMT, including the oldest (mean age, 82 years), the most sick (severe patients were not excluded, in contrast to many published studies), and those with the most comorbidities (based on a high Charlson score and high prevalence rates of bedridden and dementia in our population); (7) the outcome was critical (mortality from any cause) and not questionable (no surrogate); (8) the risk-benefit ratio was high; and (9) the cost (use of resources) for society was quite reasonable as the procedure was already routinely performed in the frequent indications currently accepted (recurrence). Modification of the recommendations would therefore result in negligible additional costs for healthcare systems.

Therefore, waiting for double-blind randomized controlled trials to update the recommendations and management of the most vulnerable and severely ill C. difficile–infected patients who are at very high risk of mortality (one of the highest risks among infectious diseases, higher than the 2014–2015 Ebola virus fatality rate (39%; www.who.int) does not seem ethical. This issue illustrates the “parachute paradigm” [42, 43]. Alternatively, we advocate confirming this benefit in nonrandomized studies, taking as controls those who refuse treatment (in our experience, a very small minority of patients with severe disease, in view of the suffering and anxiety associated with severe CDI) or with controlled before-and-after studies.

Additional studies are needed to clarify the short- and long-term complications and the few contraindications of FMT via any route (colonic FMT could be used in patients with ileus or occlusive syndrome). In such patients, medical treatment alone or surgery (perforation, peritonitis) would remain the only salvage treatments, though these therapies are associated with very poor prognoses. Overall, as suggested by Brandt et al [44], our results strongly suggest that early FMT should be proposed as a “first-line” treatment for severe CDI.

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
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