University of Messina

35th cycle International UNIME PhD
Translational Molecular Medicine and Surgery

Department of Biomedical, Dental, Morphological
and Functional Imaging Sciences

Coordinator Professor Gaetano Caramori

Academic Year 2021 – 2022

Short-term and long-term follow-up after faecal microbiota transplantation in patients with *Clostridioides difficile*

PhD Candidate
Dr Federica Giambò

Tutor
Prof. Giovanni Raimondo

Co-Tutor
Prof. Walter Fries

Tutor PhD Visiting
Prof. Giovanni Cammarota
Dr Gianluca Ianiro

1. Abstract

BACKGROUND AND AIMS: Faecal microbiota transplantation (FMT) is a highly effective therapy for recurrent *Clostridioides difficile* infection (rCDI). There is also significant evidence that FMT is safe in the short-term, but data on long-term safety are still emerging. This study aims to explore and describe long-term safety data in a cohort of patients who received FMT for rCDI and to evaluate the efficacy at 8 weeks follow-up.
METHODS: Between October 2020 and August 2022, we carried out a prospective cohort study of patients undergoing FMT for rCDI. Data on demographic and comorbidities were recorded at baseline. Then, patients were contacted 2 weeks, 2 months, 6 months (short-term follow-up), and 1-year after FMT (long-term follow-up). At each time point, symptoms and new medical diagnoses of patients were collected. Treatment success was defined as a clinical cure 8 weeks after FMT with the absence of diarrhoea and with no recurrence.
RESULTS: A total of 69 patients underwent FMT. The mean age was 68 years (range, 18–94 years old), and 34 patients (49,3%) were women. Patients underwent repeated FMT in case of failure or recurrence after the first infusion or severe clinical picture including the presence of pseudomembranous colitis. Specifically, 45 patients received a single FMT (65,2%) 18 patients received a second infusion (26%), four patients received three infusions (5,8%), one patient received four infusions (1,44%), and another patient received a sixth infusion (1,44%). At the end of the follow-up, 7 patients (10,15%) died due to causes not directly attributable to FMT, as evaluated by the experts. None of the 69 patients received a new diagnosis of autoimmune, gastrointestinal, or malignancy during follow-up. The cure rate at 8 weeks was calculated in 66 patients

(3 were lost at 8 weeks follow-up) treated by single and multiple infusion and was around 86,36% (n=57).

CONCLUSION: In our cohort of patients with rCDI, FMT appeared to be a safe procedure in short and long terms follow-up with a low risk of transmission of infections. In conclusion, no long-term adverse events or complications directly attributable to FMT were found in our prospective cohort, but an extension of monitoring could be interesting.

2. Background

2.1 *Clostridium difficile*

C.difficile is a gram-positive, obligate anaerobic, spore-forming bacillus that, under certain conditions, releases toxins and is the most common causative agent of severe nosocomial antibiotic-associated diarrhoea and pseudomembranous colitis that leads to significant morbidity and mortality worldwide (1). *C. difficile* was initially isolated in 1935 by *Hall and O'Toole* as a commensal of the microbial flora of newborns and named Bacillus difficile due to its bacillary morphology and the difficulty in isolating it in growing it in normal culture. Transmission of *C. difficile* occurs via the faecal-oral route, it occurs in 5-15% of healthy adults and can be transient (2). The transmission of infection within healthcare facilities is mainly due to environmental surfaces and the passage by direct contact with infected patients (1). Indeed, the physical proximity of a patient with CDI increases the risk of contracting the infection (relative risk interval = 1.86, confidence interval 1.06-3.28) (3). The highest contagion risk includes patients over 65 years old hospitalized in long-term facilities (4) with previous antibiotics treatment which determined a reduced immune response to *C. difficile* (5). *C. difficile* can produce both toxigenic and nontoxigenic strains but the virulence of the microorganism is linked only to toxigenic forms. Pathogenicity is dependent on the presence of three closely related diarrhoea-producing toxins known as Toxin A (TedA), Toxin B (TedB) and CDT (binary actin-ADP-ribosylating toxin), responsible for a more severe picture associated with mortality up to 30 days post-infection (6). TcdA and TcdB are characterized by a common molecular mechanism of action: inactivation of Rho GTPases through enzymatic glucosylation of a conserved

threonine residue. This leads to the depolymerization of actin resulting in cell death and activation of the inflammatory cascade that causes tissue damage, diarrhoea, and sometimes pseudomembranous colitis. On the other hand, CDT has been shown to increase the virulence of C. difficile through irreversible adenosine diphosphate-ribosylation of actin, inducing the formation of long protrusions of host cell microtubules that facilitate bacterial adhesion (7).

Specifically, TcdA and TcdB have four functional domains:

- GTD (glucosyltransferase) at the amino-terminal end;
- APD (autoprotease domain);
- Delivery domain;
- CROPS (combined repetitive oligopeptides domain) at the carboxy-terminal end.

The two toxins access into the cell through a receptor-mediated endocytosis process; in particular, through the CROPS domain, the toxin binds to the receptor that recognizes carbohydrates, glycolipids, and glycosylated proteins (8).

The expression of the two toxins is regulated by a pathogenic area called PaLoc, which codes for the two homologous toxins (TcdA and TcdB) and three proteins with gene and secretory regulation functions (TcdR, TcdC and TcdE). Several factors influence the synthesis of TcdR and consequently the activation of the TcdA and TcdB genes: the presence in the bowel of SCFAs (short-chain fatty acids) such as butyric acid favour its production, while amino acids such as cysteine and proline reduce the expression of toxins through the action of the transcriptional regulator CodY. Once secreted, the two toxins bind receptors present in the cells of the colon epithelium triggering the production of cytokines, chemokines,

the recall of neutrophils and the destruction of tight junctions, up to apoptosis (9).

In addition to direct exposure to the microorganism, another established key connection for CDI is prolonged antibiotic therapy (10). The use of broad-spectrum antimicrobials, the use of multiple antibiotic agents, and a longer duration of antibiotic therapy contribute to the increased incidence of CDI (11) (Table 1). The agents most involved in CDI are fluoroquinolones, clindamycin, penicillin and cephalosporins. The first association between C. difficile ("J strain") and ATB is linked to clindamycin and dates to the early 1990s (12). The increasingly frequent use of fluoroquinolones has been linked to *C. difficile* diarrhoea. Furthermore, the resistance to fluoroquinolones of the hypervirulent NAP1/BI/027 strain may be an important factor in determining its increase in virulence (13). The benefit-risk after interrupting antibiotic treatment is still not well understood. A case-control study with 337 patients with *C. difficile* suggested that the risk was increased during antibiotic therapy and in the three months following discontinuation of therapy; the risk was increased during treatment and in the first month after the end of the assumption of the therapy (14).

FREQUENTLY ASSOCIATED	OCCASIONALLY ASSOCIATED	RARELY ASSOCIATED
Macrolide	Clindamycin	Sulfonamides
Fluoroquinolones	Trimethoprim	Chloramphenicol
Aminoglycosides	Tetracyclines	

Table 1. Antimicrobials responsible for C. difficile diarrhoea

Antibiotics decrease colonization resistance, thus providing input for colonization by intestinal pathogens. In particular, in the pathophysiology of *C. difficile*, a reduction of Bacteroides and Firmicutes phyla was noted following the prolonged use of antibiotics (15).

Despite the growing challenge of antibiotic resistance, their use is recommended by international and national guidelines in initial and recurrent episodes of CDI, preferring the use of standard antibiotics such as Vancomycin, Metronidazole and Fidaxomicin (16). There are various treatment options for CDI. Antibiotic therapy is the standard first-line therapy for CDI and the choice of antibiotic therapy is tailored to the severity of the disease presentation. Metronidazole may be administered orally or intravenously, whereas vancomycin may be given per os or rectum. The rationale for why vancomycin is not used intravenously in the treatment of CDI is that it insufficiently penetrates into the intestinal mucosa. Worthy of mention, Fidaxomicin is a novel macrocyclic antibiotic, administered orally, with a narrow spectrum of activity (17).

Another dominant factor that, according to epidemiological studies, increases the risk of CDI includes the overuse of proton pump inhibitor (PPI) therapy (18), which has resulted in a drug safety communication issued by the U.S. Food and Drug Administration (FDA) (19).

Although the scientific evidence supports an association between PPI use and the incidence of CDI, PPIs remain the most effective drug in their therapeutic class, thanks to their reduced side effects and low tolerance (20).

Impairment of the immune response also plays a role in the predisposition to infection (21). Other demonstrated risk factors for CDI are an indication of patients' underlying susceptibility: advanced age (22), suppressed gastric acidity (23), low albumin (<3.5 g/dl, <2.5 g/dl associated with

severe disease) (24), and various underlying disease severity such as gastrointestinal surgery, nasogastric tube feeding, and concomitant gastrointestinal diseases (25). Numerous variables influence the possibility of the onset of CDI. Several scores have been proposed to predict patient prognosis but a clinically useful tool for stratifying resource use has not been determined. *Horn's index*, a severity score based on the underlying clinical disease, reliably predicts patients at high risk for CDI (26).

However, clinical pictures determined by *C. difficile* can differ widely and display variable symptoms. CDI can cause a spectrum of manifestations from asymptomatic, moderate watery diarrhoea with self-limiting benign evolution to severe pictures such as fulminant pseudomembranous colitis, toxic megacolon, intestinal perforation, and septic shock. Although signs and symptoms usually develop within 5-10 days of starting a course of antibiotics. However, the range in which symptoms can occur is as early as the first day or up to three months later. The cardinal symptom of CDI is watery diarrhoea (at least five bowel movements with liquid or unformed stools during the last 36 h) defining a condition called *C.difficile* -associated diarrhoea (CDAD). The other most common signs and symptoms of mild to moderate include lower abdominal cramping, abdominal distention, and tenderness, low-grade fever, and nausea (27).

People who have life-threatening diseases are usually dehydrated and may need to be hospitalized. Clinical manifestations of severe colitis include liquid diarrhoea as often as 10 to 15 times a day, diffuse and unspecific abdominal pain, weight loss, fever, hypovolemia, lactic acidosis, hypoalbuminemia, elevated creatinine, and marked leukocytosis, which may be very severe (28).

Blood or pus are frequently revealed in the stool, and pseudomembranes in the colon are present in less than 25% of the most severe episodes. Recurrent CDI is defined as the eradication of *C. difficile* during appropriate therapy, followed by the recurrence of symptoms within two to eight weeks of ending treatment. In about 20% of cases (from 5 to 47%), a first recurrence occurs, and of these, 40-45% undergo a second recurrence; in general, 60-65% of patients have multiple recurrences (29). Re-exposure to *C. difficile* has a higher probability of developing an infection. In contrast, patients previously colonized with *C. difficile* are more likely to remain asymptomatic during hospitalization (30). The magnitude of this difference was illustrated in a prospective review of 810 hospitalizations, including 618 patients with new *C. difficile* exposure and 192 patients with prior *C. difficile* colonization (31). The pathogenesis of relapses would seem to be attributable to factors related to the *C. difficile* strain, alterations in the normal intestinal bacterial flora caused by antibiotic treatments and / or an altered immune response on the part of the host (32).

CDI can have many facets and has been established as a substantial burden having a high economic impact on healthcare systems worldwide. A systematic review conducted in 2019 led by *Balsells et al.* analyzed the rate incidence in 41 countries through 229 publications (33). Most of the data come from databases in the United States. The cumulative incidence of CDI reported in individual studies ranged from 1.12 to 631.80 per 100 000 population per year. Moreover, in the last decade, there has been emerging unexpected data on the occurrence of CDI in non-hospitalized patients, which is of interest mainly to young, healthy, and not exposed to antibiotic therapy patients (34). *Eyre et al.* led a molecular epidemiological study using whole-genome sequencing to recognise

exposure and transmission of *C. difficile* concluding that person-to-person transmission in hospitals accounted for almost a third of infections (35).

During the COVID-19 pandemic, several factors changed the incidence of CDI in a short time. In June 2022, *Vendrik et al.* published a paper comparing data recorded through the "Dutch sentinel CI surveillance" analyzing CDI incidence between 2015 and 2020 (36).

There was an increase in the rate of severe CDI during the second wave of the COVID-19 pandemic, potentially due to delays in diagnostic testing and restrictions on hospital referrals for patients with community-onset CDI. Subsequently, the improvement of hand hygiene, social distancing, and restricted hospital referral, may have had a positive influence on the epidemiology of CDI, reducing the incidence rate.

2.2 Gut Microbiota

At the root of CDI pathogenesis is the disruption of the microbiota. The human intestinal microbiota is a morpho-functional entity made up of more than a thousand commensal microorganisms, including bacteria, phages, archaea, fungi, viruses, and eukaryotic microorganisms (37). The gut microbiome (GM) comprises the totality of microorganisms, bacteria, viruses, protozoa, and fungi, and their collective genomes inhabiting our gut (37). The unique composition of the microbiota is different in each individual, and its variability depends on several factors, including the age of the host organism, genetic factors, and environmental factors (39,40). The first imprinting occurs already at birth when each human's gut microbiota is shaped and then varies considerably in the following years depending on infant transitions, quality of gestation (41), type of birth (42), milk composition (42), method of lactation feeding (43), weaning period (44), dietary habits (45). GM is uniquely characterized by clusters of bacteria known as enterotypes (46). Each enterotype is characterized by three main bacteria clusters: Bacteroides (enterotype I), Prevotella (enterotype II), or Ruminococcus (enterotype III). In the first years of life, the predominant bacterial species are Bifidobacteria. As the bacterial component grows, it increases both in terms of diversity and functionality, reaching a high complexity in adulthood. Through the innovative methods of NGS (next-generation sequencing), the four most represented microbial phyla have been identified among the approximately 35.000 bacterial species. The dominant gut microbial phyla are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia, with the two phyla Firmicutes and Bacteroidetes representing 90% of GM. In old age, the gut microbiota changes again, resulting in a decrease in terms of diversity of species and a modification of the balance between them.

Bacteroidetes and Proteobacteria increase compared to Firmicutes and Bifidobacterium (47). The commensal bacteria present in the human faecal flora are represented by two main groups of Firmicutes, divided into *Clostridium coccoides* (Clostridium cluster XIV) and *Clostridium leptum* (Clostridium cluster IV), which are butyrate producers, and by the Cytophaga-Flavobacterium-Bacteroides group (CFB) (48).

The bowel hosts the most significant number of bacterial species (about 70%). The predominant phyla in this district are the Firmicutes and the Bacteroidetes. In low concentrations, the human colon is also colonised by pathogenic microorganisms such as *Campylobacter jejuni*, *Salmonella* spp, *Vibrio* spp, *Escherichia coli* and *Bacteroides fragilis*. The phylum Proteobacteria is poorly represented, and in association with an abundance of Bacteroides, Prevotella and Ruminococcus correlate with a balanced and healthy intestinal microbiota.

GM offers many benefits to the guest. It confers resistance to colonization by pathogens, develops the host immune response, and has important metabolic functions (49). This occurs through various mechanisms including the production of inhibitory molecules, nutritional competition, and stimulation mechanisms of local innate lymphoid cells (ILC), myeloid cells, or T and B lymphocyte responses. ILCs have been implicated in the protection against various agents. intestinal pathogens and ILC1s protect against *C. difficile* through the production of interferon (IFN) -γ (50).

Modifications of the GM, defined as dysbiosis, and the modulation of the innate immune response play a fundamental role in CDI and eventual aggravation of the infection.

C. difficile may be a non-pathogenic commensal in the human intestine (51), but it is able to modulate the innate immune response in the intestine.

Innate immune mechanisms targeting *C. difficile* toxins include endogenous microbial flora, the mucosal barrier, intestinal epithelial cells, and the mucosal immune system. Furthermore, CDI triggers the release of several proinflammatory mediators (cytokines, chemokines, and peptides) and the recruitment and activation of several innate immune cells *C. difficile* toxins activate both surface and intracellular sensors, which are part of innate immunity, including inflammasome and TLR4, TLR5, and Nod1 (52). TLR4- and MyD88-dependent signaling pathways produce a major inflammatory response (53). The deficiency of these pathways increases the bacterial load and worsens the course of the disease. Mice lacking TLR4- and MyD88-dependent activity was found to be more susceptible to infection and more exposed to the disease than wild-type mice (54). Neutrophils appear to have the ability to prevent the spread of bacteria through the damaged mucosa (55). The activation of innate immunity and the release of cytokines and chemokines are followed by an intense infiltration of neutrophils at the local level. This infiltration of neutrophils constitutes one of the main pathological findings following CDI. Indeed, the induction of neutropenia in rats was associated with less severe disease. It suggests that intestinal dysbiosis and the altered innate immune response are crucial players in triggering the colonization of C. difficile and related symptoms. An underlying condition of dysbiosis results in reduced resistance to colonisation, which encourages the growth of microbes such as *C. difficile* to also modify the structure of the microbiota in the individual (56). Frequent use of ATB leads to a steady increase in antibiotic resistance in the microbiome (57).

2.3 *Clostridium difficile* infection

CDI is a condition characterised by acute onset of diarrhoea with documented positivity for *C. difficile* toxin or demonstration of pseudomembranous colitis via colonoscopy or histopathological findings (6) (Table 2).

The symptomatologic bouquet of CDI includes from asymptomatic presentation to mild, moderate, or self-limiting diarrhoea to pseudomembranous and sometimes fatal colitis (58).

Several studies have shown that more than 50% of hospitalised patients with C. difficile are asymptomatic carriers, possibly due to natural immunity.

In the case of symptomatic disease, symptoms of *C. difficile* infection appear after approximately 2-3 days (59).

C. difficile diarrhoea (characterised by ≥3 Bristol Stool Chart type 6 or 7 bowel movements in 24 hours) may be associated with mucus or occult blood in the stool, but melaena or sharp hematochezia are rare. Fever, cramps, abdominal pain, and peripheral leukocytosis are common but are seen in less than half of patients. Extra-intestinal manifestations, such as arthritis or bacteremia, are infrequent. *C. difficile* ileitis or pouchitis has rarely been recognised in patients undergoing total colectomy (for complicated CDI or other indications). Patients with severe disease may develop abdominal pain and distension but with little or no diarrhoea.

Complications of a severe infection include dehydration, electrolyte disturbances, hypotension, hypoalbuminemia, toxic megacolon, intestinal perforation, kidney failure, sepsis, and even death.

Under normal conditions, many bacterial species dominating our gut compete and outclasses *C. difficile.* Broad-spectrum antibiotics eliminate

bacterial flora, allowing *C. difficile* spores to proliferate without competition with non-pathogenic bacterial species (60).

Category	Clinical and Laboratory signs	Associated Risk Factors
Mild to Moderate	Diarrhoea without systemic signs of infection, white blood cell (WBC) count <15.000 cells/mL, and serum creatine <1.5 times baseline.	Antibiotic use, previous hospitalisation, longer duration of hospitalisation, use of proton pump inhibitors (PPI), receipt of chemotherapy, chronic kidney disease, and presence of a feeding tube.
Severe	Systemic signs of infection and/or WBC count ≥ 15.000 cells/mL, or serum creatine ≥ 1.5 times the premorbid level.	Advanced age, infection with BI/NAP1/027 strain.
Severe/ complicated	Systemic signs of infection include hypotension, ileus, or megacolon.	See above, plus recent Surgery, history of inflammatory bowel disease (IBD), and intravenous treatment.
Recurrent	Recurrence within 8 weeks of completing treatment for CDI.	Patient age ≥ 65 yo, concomitant antibiotic use, significant comorbidities, concomitant use of PPI, and increased initial disease severity.

Table 2. Clinical manifestation of CDI

2.3.1 Laboratory diagnosis

The diagnosis of CDI is based on a combination of signs and symptoms, confirmed by microbiological evidence of *C. difficile* toxins in the absence of other causes; or on histopathological findings or colonoscopy, diagnostic for pseudomembranous colitis (16).

Different approaches can be used in the laboratory diagnosis of CDI; also, the search for indices of inflammatory reaction (leukocytes, lactoferrin) is often positive (although the data are still insufficient to comply with the ESCMID recommendations), however, the best laboratory test has not yet been defined.

Diagnostic tests for *C. difficile* can be divided according to the detection target:

- toxigenic culture is a selective culture method for toxin-producing strains of C. difficile; A selective agar medium is used which is then incubated under anaerobic conditions. subsequently, the microorganism is identified according to morphological and organoleptic criteria. This method has high sensitivity and low specificity. In addition, the long culture and identification times make it a non-usable test in screening.
- The CCNA (C. difficile cytotoxin neutralization assay) directly identifies the toxin in the stool; after the faecal material has been filtered and applied to a cell monolayer it is possible to observe the cytopathic effect caused by toxins; At the same time, a test with antibodies that neutralize C. difficile toxins is performed to ensure that the cytopathic effect is not caused by other substances. It is a long-term method with no standardization, although it has high specificity.

• EIA (Toxin A and B Enzyme Immunoassays) uses monoclonal or polyclonal antibodies to detect C. difficile toxins; the sensitivity values range between 60-92%, and for this reason, it is not recommended to use it as the only test.

• GDH is a test that detects the presence of glutamate dehydrogenase; an antigen expressed on the cell wall of C. difficile. Sensitivity to GDH is often used as a screening test although up to 10% of patients with toxigenic microorganisms can escape with this method. This test, therefore, represents the first step of the 2 or 3-step diagnostic algorithm that combines it with a toxin test and / or a molecular test for the identification of the toxin gene.

• NAAT (nucleic acid amplification test) is a molecular method that detects specific genetic sequences, so it is a useful test for detecting toxigenic strains of C. difficile. Several observational studies have shown that the exclusive use of molecular methods leads to an "overdiagnosis" of CDI.

The European Society of Clinical Microbiology and Infectious Disease (ESCMID) recommends the use of a two or three-step algorithm for diagnosis that considers aspects such as costs, time, specificity, and sensitivity (6) (Table 3).

The first step is characterized by the choice of an extremely sensitive test (GDH test) which aims to screen subjects positive for *C. difficile*. If the test results are negative, the diagnostic process is interrupted, and the sample is considered negative for *C. difficile*. In the case of a positive GDH test, proceed to the second step (EIA) for the detection of the toxin. In case of negativity to the EIA, proceed with the molecular methods (NAAT).

TEST	SENSIBILITY	SPECIFICITY	AVAILABILITY	COST	USE OF THE TEST
Culture of *C. difficile*	LOW	MODERATE	LIMITED	5-10$	Not diagnostic use
Toxigenic culture	HIGH	LOW	LIMITED	10-30$	Epidemiological tool, limited diagnostic use
CCNA	HIGH	HIGH	LIMITED	15-25$	Limited diagnostic use
GDH	HIGH	LOW	WIDE	5-15$	Limited diagnostic use
Test EIA	HIGH	MODERATE	WIDE	5-15$	Identifies A + B toxins; lower sensitivity
NAATs	HIGH	HIGH	WIDE	20-50$	Used only for acute illness; possible false positives

Table 3. Diagnostic tests for CDI

2.3.2 Management and treatment

In order to clearly manage the treatment of CDI, it is essential to recognize the grade of the disease. The severity of the disease is classified as follows:

- mild illness: CDI with diarrhoea as the only symptom;
- moderate illness: CDI with watery diarrhoea, and abdominal pain but no other symptoms/signs;
- severe disease: CDI with (or developing during the course of the disease) hypoalbuminaemia (serum albumin <3 g / dl) and one of the following: white blood cells (WBC) ≥ 15,000 cells / mm3 or abdominal pain without being a criterion for complicated disease;
- complicated infection: the patient has or develops at least one of the following signs or symptoms: admission to the intensive care unit, hypotension with or without the need for vasopressors, fever ≥ 38.5 ° C, ileus (intestinal paralysis), or significant abdominal distension, altered mental status, WBC ≥ 35,000 cells / mm3 or <2,000 cells / mm3, serum lactate levels> 2.2 mmol / L, or any evidence of organ failure.

Recently a multidisciplinary group representing the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) has proposed practical guidelines on the management of Clostridioides difficile infection (CDI) in adults that specifically addresses the use of fidaxomicin and bezlotoxumab for the treatment of CDI (61).

According to the guidelines, the first episode should be treated with the standard dose fidaxomicin for 10 days, alternatively, oral standard dose oral vancomycin can be used for 10 days.

In case of the first relapse in recurrent CDI guidelines propose to use of standard-dose fidaxomicin or higher-strength vancomycin.

In the case of multiple recurrences, the guidelines include several treatment options including faecal microbiota transplant (FMT). It is worth noting that FMT is recommended for patients who have already received appropriate antibiotic treatment for at least two relapse episodes (or three episodes of CDI).

In the dramatic case of fulminant CDI, characterized by hypotension or shock, ileus or megacolon, the recommended treatment is preferably antibiotic treatment however it is important to emphasize that there is no current evidence to support the use of fidaxomicin.

2.4 Faecal Microbiota Transplantation

Faecal microbiota transplantation (FMT) is the infusion of faecal material from a healthy donor into the gastrointestinal tract (GI) of a recipient to treat a specific disease related to dysbiosis changing the microbiota composition and confer a health benefit.

The first documented use of FMT or “yellow soup" for treating gastrointestinal diseases dates back to about 1500 years ago in China. Also, Bedouins have a long tradition of using camel faeces as a remedy for dysentery.

However, FMT was first applied in traditional medicine only in 1958, when Ben Eiseman et al. successfully treated 4 patients with pseudomembranous colitis using faecal enemas (62). A considerable body of evidence has shown that FMT is highly effective in the treatment of severe, refractory, and recurrent CDI (63,64). FMT demonstrated a high efficacy rate in randomised controlled trials (65), in which FMT superiority was documented over vancomycin and in systematic reviews and meta-analyses (66, 67) showing a surprisingly cure rate of average resolution of this pathology which ranges from 85% to 94%, and at the same time a good result in terms of safety for patients (66), which is much superior to prolonged anti-microbial therapy with 20–30% success rates (68).

In the last decades, FMT has moved from an offbeat strategy to a globally established therapy, regulated by international guidelines (69). FMT was approved by both the European Society for Microbiology and Infectious Disease (ESCMID) and, the American College of Gastroenterology (ACG) recommend FMT for preventing rCDI not resolved with antibiotic regimens. The ESCMID panel agreed that FMT is best reserved for patients who have experienced at least two episodes of rCDI (6). Other

applications for FMT include the management of refractory CDI and, eventually, even primary CDI (70). Nevertheless, the efficacy of FMT seems to be higher for recurrent than for refractory CDI (71). A large body of data shows that when guidelines for donor screening are followed, FMT is a safe treatment in the short term, with mostly transient, mainly gastrointestinal side effects and risks from endoscopic procedures (67, 72).

Notably, data concerning the potential long-term impact following FMT is currently limited in the literature. Rarely autoimmune, metabolic, and psychiatric diseases have been reported after FMT (73). Patients receiving FMT for rCDI are often multimorbid with reduced life expectancy, and it is difficult to measure the long-term risks and the potential for adverse events (AEs) related to FMT.

In this ongoing, prospective, observational single-centre cohort study, we aimed to estimate the long-term safety of FMT in patients with recurrent and refractory CDI.

3. Materials and Methods

3.1 Aim of the study

The aim of the study was to estimate the efficacy at 8 weeks after FMT and short and long-term safety after FMT in recurrent and refractory CDI. Patients were followed up via face-to-face visits or telephonically. During routine follow-up, patients were asked about symptoms of CDI recurrence (diarrhoea, bloating or abdominal pain) and frequency of stools. Also, patients were instructed to call or visit investigators in case of recurrence of diarrhoea, bloating or abdominal pain. Safety outcomes included patient symptoms, infections, hospitalisations, deaths, and changes in current medical conditions or the development of new medical conditions.

3.2 Study design

A single-centre, prospective cohort study was conducted at the Fondazione Policlinico Universitario "A. Gemelli", a tertiary care centre based in Rome, Italy, in patients undergoing FMT for rCDI between October 2020 and August 2022.

3.2.1 Patients

Patients were admitted to our University Hospital with a diagnosis of rCDI, defined as watery diarrhoea, within 8 weeks of a previous CDI episode with a positive stool *C. difficile* test. The indication for FMT was considered on a case-by-case basis, considering factors such as the number and severity of previous episodes of CDI; previous treatment modalities administered and possible alternative strategies; comorbidities; and the safety, feasibility, and practicality of the FMT administration, given the specific complexities presented by the COVID-19 pandemic.

Baseline characteristics of recipients of FMT were collected in terms of demographic, medical history, concomitant disorders, and medications. The presence of comorbidities was analyzed and used to calculate the Charlson comorbidity index (CCI) (74). An accurate list of comorbidities has been drawn according to the last International Classification of Diseases (ICD-11) adopted by the 72nd World Health Assembly in 2019 and effective from 1st January 2022.

Exclusion criteria were refusal of informed consent, pregnancy, and patients aged <18 years. We excluded patients with the first episode of CDI, as FMT is not recommended in this situation (69), and those considered unfit for FMT to avoid selection bias.

3.2.2 Donors

The management of the selection and recruitment of donors was performed at the Fondazione Policlinico Universitario "A. Gemelli", following protocols recommended by international guidelines (75).

The preliminary questionnaire (Table 4) investigated critical landmarks of the possible donor: known history or risk factors for infectious diseases; history of gastrointestinal, metabolic, and neurological disorders; use of drugs/substances that can alter the intestinal microbiota.

The presence of infectious diseases was excluded analyzing:

- Known history or exposure to HIV, HBV or HCV, syphilis, human T-lymphotropic virus I and II, malaria, trypanosomiasis, tuberculosis;
- Known systemic infection not controlled at the time of donation;
- Use of illegal drugs and / or substances;
- Sexual risk behaviours (occasional/anonymous sexual intercourse/contact, high-risk sexual behaviour with prostitutes, drug addicts, HIV positive subjects, hepatitis, syphilis; prostitution; history of sexually transmitted disease);
- Subject undergoing tissue/organ transplant;
- Previous (<12 months) transfusion of blood/blood components or administration of blood products;
- Recent (<6 months) accidental syringe puncture;
- Recent (<6 months) execution of tattoos, piercings, earrings, acupuncture;
- Recent medical treatment in conditions of poor hygiene (e.g. field hospitals, medical treatments in makeshift disorders, non-standard medical treatments);

• Risk of transmission of diseases caused by prions (Transmissible Spongiform Encephalopathies);

• Recent parasitosis or infections with rotavirus, Giardia lamblia and other microbes with the gastrointestinal implication;

• Recent trips (<6 months) to tropical countries, countries with a high risk of communicable diseases or traveller's diarrhoea;

• Recent (<6 months) history of live attenuated virus vaccination, if there is a risk of transmission;

• Work as a health worker;

• Work in contact with animals (to exclude the risk of transmission of zoonotic infections);

Any presence of gastrointestinal, metabolic, and neurological disorders was sifted:

• History of irritable bowel syndrome, chronic inflammatory bowel diseases, chronic functional constipation, celiac disease, and other chronic gastrointestinal disorders;

• History of chronic and systemic autoimmune diseases with gastrointestinal involvement;

• History or high risk of gastrointestinal cancer (including polyposis);

• Recent onset of diarrhoea or hematochezia;

• History of neurological or psychiatric pathologies;

• Overweight and obesity (body mass index> 25);

The assumption of any medication was recorded:

• Recent (<3 months) exposure to antibiotics, immunosuppressants, and chemotherapy;

• Chronic therapy with proton pump inhibitors.

Drugs that can alter gut microbiota
Use in the last three months of:
• Systemic antimicrobial drugs
• Immunosuppressant agents
• Chemotherapy
Daily use for over three months:
• Proton pump inhibitors
Disorders potentially associated with the disruption of GM:
• Personal history of chronic GI disease, including functional GI disorders, IBD, coeliac disease; other chronic GI diseases or Recent abnormal GI symptoms (eg, diarrhoea, haematochezia, etc.)
• Personal history of cancer, including GI cancers or polyposis syndrome, and First-degree family history of premature colon cancer
• Personal history of systemic autoimmune disorders
• Obesity (BMI >30) and/or metabolic syndrome/diabetes
• Personal history of neurological/neurodegenerative disorders
• Personal history of psychiatric/neurodevelopmental conditions
Know history or risk behaviors for infectious disease
• History of HIV, hepatitis B or C viruses, syphilis, human T-lymphotropic virus I and II
• Current systemic infection
• Use of illegal drugs
• High-risk sexual behaviour
• Previous tissue/organ transplant
• Recent hospitalization or discharge from long-term care facilities
• High-risk travel/engaged in medical tourism
• Needle stick accident in the last six months
• Body tattoo, piercing, earring, acupuncture in the last six months
• Enteric pathogen infection in the last two months
• Acute gastroenteritis with or without confirmatory test in the last two months
• History of vaccination with a live attenuated virus in the last two months

Table 4. Preliminary interview for donor selection

The subjects found fitted by the questionnaire subsequently performed a panel of bio humoral tests on serum and faeces (Table test). Most of these tests were recommended for each potential donor, while some tests were recommended for specific conditions, in the physician's judgment.
Blood tests included:

• Serology for Cytomegalovirus, Epstein-Barr virus, HAV, HBV, HCV, HEV, Syphilis, HIV 1 and 2, Entamoeba histolytica;

• CBC, ESR, PCR, albumin, creatinine, electrolytes, transaminases, bilirubin, GGT, alkaline phosphatase;

Faecal tests included:

• *C. difficile* toxin;

• Detection of enteric pathogens, including Salmonella, Shigella, Campylobacter, Escherichia coli O157 H7, Yersinia, vancomycin-resistant Enterococci, methicillin-resistant Staphylococcus aureus, multidrug-resistant Gram-negative, Norovirus, protozoa (including Blastocystis hominis, Dientamoa, fragileni) / or rapid test for G. lamblia and Cryptosporidium parvum;

• Faecal occult blood test (FOBT).

Blood exams
• Complete blood cell count
• Liver enzyme (Aminotransferases)
• Bilirubin
• Creatinine
• C-reactive protein
• Serology for Hepatitis virus (HAV, HBV, HCV, HEV) and Human immunodeficiency virus (HIV)
• Nematodes (Strongyloides stercoralis)
Stool exams
• Clostridium difficile (GDH, Toxin A/B Enzyme Immunoassay)
• Microscope and molecular examination for Helminths and pathogenic Protozoa as Entamoeba histolytica, Blastocystis hominis, Dientamoeba fragilis Giardia lamblia, Cryptosporidium spp, Cystosospora belli, Cyclospora cayetanensis and Microsporidia
• Culture examination for Multi Drug-resistant bacteria (MDRB)
• Culture and molecular examination for enteric pathogens: Salmonella spp., Shigella spp., Campylobacter spp, Shiga toxin-producing E. coli, Entero-Pathogenic E.coli (EPEC), Entero-Toxigenic E.coli (ETEC), Entero-Invasive E.coli (EIEC), Entero-Aggregative E.coli (EAEC), Y. enterocolitica, Aeromonas spp., Plesiomonas shigelloides and Vibrio cholera, C. difficile toxin-B
• Molecular examination for Norovirus, Rotavirus, Adenovirus, Sapovirus, Astrovirus
• Helicobacter pylori faecal antigen

Table 5. Donor blood and stool analysis

A further questionnaire was administered to the donors on the day of the donation to ensure that no events may have occurred in the interval between the acquisition of the laboratory results and the donation determining the current ineligibility for recruitment.

The second questionnaire aimed at identifying the following risk factors:

- Appearance of diarrhoea, nausea, vomiting, abdominal pain, jaundice, and other gastrointestinal symptoms
- Onset of disease, or, in general, fever, sore throat, lymphadenopathy
- Use of antibiotics or other drugs that can alter the intestinal microbiota
- Intake of potentially harmful substances
- New sexual partners, high-risk sexual behaviours, travel abroad (particularly in tropical environments), contact with human blood
- Appearance of diarrhoea among people with whom one is in frequent contact in daily life. A positive response to even one of the questions in the questionnaire will result in the person being not enrolled as a donor.

Since the onset of the COVID-19 pandemic, donor selection represented a fundamental challenge given the implementation of FMT programs worldwide. Knowing the donor's risk of COVID-19 infection, medical history and laboratory tests were required on the day of donation. The US Food and Drug Administration and the Italian National Transplant Center (77) have provided appropriate and clear protocols for the measures that should be adopted and readjusted by local health systems as our study group (78).

In the era of COVID-19, the questionnaire administered to screen potential donors, was carried out via telemedicine in specific conditions to avoid unnecessary exposure of potential donors to the hospital. In those cases,

the donor health questionnaire was submitted first to the candidate donor electronically and the interview was conducted through a video / telephone consultation.

The questionnaire specifically included whether the donor has been diagnosed with laboratory-confirmed SARS-CoV-2 infection or has been exposed to individuals with suspected or confirmed COVID-19 infection. As suggested by the Center for Disease Control and Prevention (CDC) (79), it is necessary to evaluate if the potential donor has or recently has manifested symptoms associated with COVID-19 infection such as fever, cough, dyspnea, chills, anosmia or ageusia, sore throat, muscle aches that cannot be explained alternative diagnosis (89). All potential donors who are identified as not at risk by the questionnaire should undergo laboratory tests. At least one nasopharyngeal swab and reverse transcription polymerase chain reaction (RT-PCR) assay and serology are used for the detection of SARS-CoV-2 (81). All potential donors who tested positive were temporarily excluded from donation, informed of the result, and advised to take precautionary measures against transmission. In this case, the donor was excluded for 30 days and then re-tested with a questionnaire and molecular stool testing.

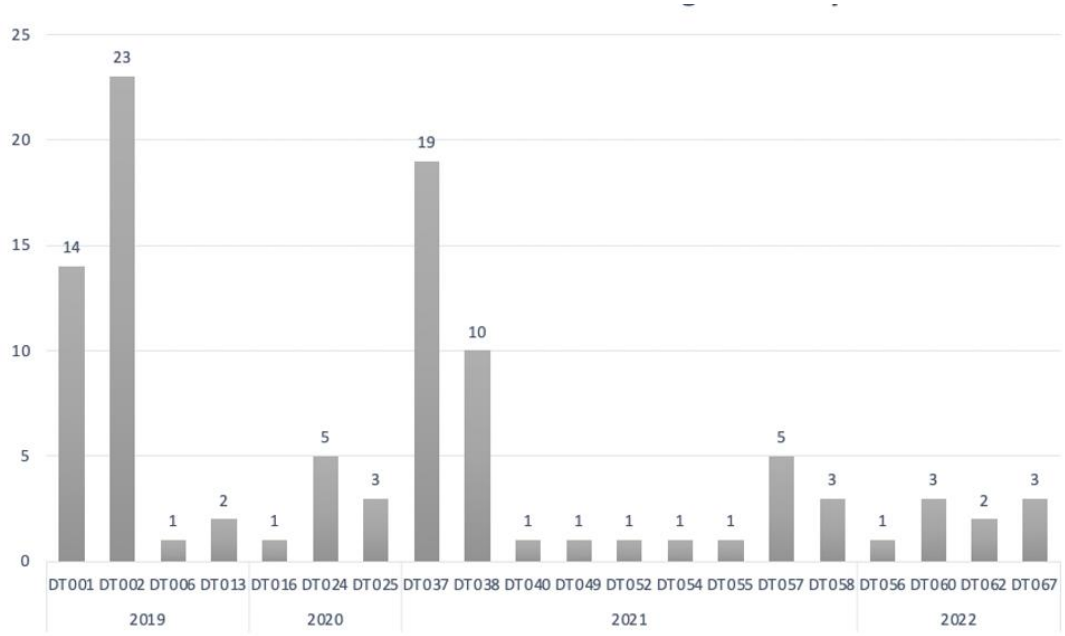
Remarkable, the shedding of SARS-CoV-2 RNA in faeces and the recognition of GI symptoms in infected subjects have been documented by several studies (82) raising major concerns about potential transmission of SARS-CoV-2 via the faecal–oral route. For this reason, the team of microbiology laboratory of our hospital (L.M.) evaluated two IVD marked assays, namely the Seegene Allplex™ SARS-CoV-2 and SARS-CoV-2/FluA/FluB/RSV, for the detection of SARS-CoV-2 RNA in the stool of potential FMT donors (83).

Potential donors who passed the questionnaire and laboratory testing described were contacted to provide faecal material. A dedicated toilet was reserved for stool collection, and the area was cleaned after each donation. If it was not technically possible, the stools were collected at home. In this case, collected stools should be kept at a temperature of about 4 °C and mandatory delivered to the stool bank within 6 hours. Therefore, clear security measures have been recommended by precedents guidelines.

According to our data, we made use of 19 donors for 100 infusions, enrolled from 2019 to 2022 (Graph 1).

As we established, the pandemic has raised several issues concerning donor recruitment, and the availability of faecal material.

From May 2019 to December 2019, 13 donors were enrolled, approximately 2 donors for a month. Nevertheless, since the beginning of the pandemic we have noted many obstacles in the recruitment of donors therefore from January 2020 to December 2020 we enrolled approximately 1 donor per month (13 donors in a year). A stackable picture was expected in 2021, instead, the availability of clear directives on recruitment and management of FMT determined a resumption in the recruitment processes even during the Covid-19 emergency. Therefore, from January 2021 to December 2021, we enroll 22 donors in a year and, even better, from January 2022 to August 2022 approximately 3 donors for a month were enrolled by our Team.



Graph 1. Donation rates from donors during our survey

3.2.3 Manufacturing of Faecal Infusate

The material collected at the donation was immediately delivered to the microbiology laboratory of our hospital (L.M.) following manufacturing protocols recommended by international guidelines. Faecal material was accepted, identified, registered, and managed by the dedicated health staff. In particular, the room used for processing faecal material is suitable and equipped with adequate equipment.

The samples of faecal material being processed are provided with a legible and indelible label that shows the unique code of the donor and the date of collection and processing.

To avoid contamination, the sample is handled with sterile microbiological techniques. Glycerol is added to a final concentration of 10% of the faecal material collected. A minimum of 50 g of faeces and 150 mL of sterile saline solution were used for each sample.

Each material is identified, registered, and immediately frozen at –80 ° C. The acceptable storage temperature range is ± 5 ° C. The storage procedure evaluates the temperature excursion and the duration of the out-of-range and eliminates the products if they are exposed to temperature excursions that impact the quality of the final product. The material is stored for a maximum of 6 months. After this deadline, if not used, the sample is eliminated by company procedures to manage hospital waste. In the event of a release, however, the associated sample rate is kept for 10 years for any checks in the event of adverse events.

As concerns possible COVID-19 infection, SARS-CoV-2 RNA-positive stool infectivity is still unclear. Preliminary data suggest that viral shedding in stool lasts about 2-4 weeks (84), gastrointestinal symptoms often anticipate respiratory symptoms and viral shedding in the stool may delay despite respiratory clearance samples (85). The deriving solution

was blended, and the supernatant was strained and poured into a sterile container. For frozen samples, glycerol was added up to a final concentration of 10% before freezing and samples were stored at 80 °C. On the day of faecal infusion, frozen infusates were thawed in a warm (37 °C) water bath.

3.2.4 Faecal Infusion Procedure

The faecal infusion procedure included the following steps: a 3-day pretreatment with oral vancomycin (250 mg per os 4 times a day); bowel cleansing with 2 L of macrogol (SELG ESSE) per day for 1 or 2 days (depending on the clinical condition of the patients); and faecal infusion from healthy donors by colonoscopy, as previously described. Evaluated each patient, appropriate Informed Consent Form was signed and dated by the patient and the member of the team taking the consent.

In the Endoscopy Room, the patient was positioned in a comfortable position on the left side and a choice of sedation was discussed with the endoscopist before the procedure. Generally, the most used routes for FMT are endoscopic ones, in particular colonoscopy. Several systematic reviews and meta-analyses have revealed that the rate of successful FMT is significantly higher in patients who received FMT via colonoscopy than in other delivery routes (63, 67).

FMT via colonoscopy has the advantage of re-colonizing the entire colon with healthy bacteria. The colonoscope allows the endoscopist to directly assess the grade of inflammation and select preferential sites for stool infusion (86). Additionally, bowel lavage can reduce the existing pathogenic content and facilitate the colonization of healthy donor microbiota (87). On the other hand, endoscopic procedures can potentially facilitate the transmission of the virus to both healthcare professionals and patients. For these reasons, guidelines on gastrointestinal endoscopy and staff use have been published regarding protective equipment (PPE) for these procedures in the environment of COVID-19 (88). When FMT is administered via colonoscopy, intestinal lavage is essential. A good intestinal toilet guarantees good visibility of the operator in the intestine

eliminates the presence of solid faecal material or food residues or drugs, and above all facilitates the engraftment of transplanted microorganisms.
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.
During the procedure, strict recommendations were provided to minimize the risk before, during and after the procedure to be followed. The number of healthcare professionals present during the procedure was minimized and those present wore full PPE, including N95 masks and screens / protective goggles, gloves, and hair nets.
The faecal samples were delivered through the operative channel of the scope after reaching the more proximal point of the large bowel, using 50-mL syringes filled with the solution at the time of colonoscopy. The volume administered was between 200 mL and 280 mL. After completing the infusion, the channel of the scope was flushed with 20 mL of normal saline. During the insertion and removal of the colonoscope, operators were able to assess the presence of pseudomembranes, presence of polyps and other inflammatory signs of the large bowel. On average, the infusion procedures were performed within 10 min, and the tube was removed after the infusion. Finally, patients were monitored in the recovery room of the endoscopy centre on their right side for 2 - 3 hours after the procedure for any side effects.
No further *C. difficile* toxin tests were indicated and if the patient developed a new episode of diarrhoea within 28 days after FMT, it was clinically assessed case by case.

3.3 Short-term follow-up

The short-term safety of FMT in patients with rCDI is thoroughly established from the increasing number of patients treated worldwide. Participants were followed up at 2 weeks through face-to-face outpatients visits at the Fondazione Policlinico Universitario “A. Gemelli” by the Gastroenterologists of the Microbiota Study Group. The survey protocol allowed these follow-up data to be collected either at clinic visits, via mail, or through telephone interviews. Due to the Covid-19 pandemic, all health facilities have drastically reduced elective activities both to avoid potential transmission of the virus and to responsibly focus human resources on managing Covid-19 (89). Treatment success was defined as a clinical improvement after 8 weeks after FMT (1). Individuals with a *C. difficile* toxin B DNA positive stool sample during follow-up but without diarrhoea were classified as treatment success. Treatment failure was defined as the presence of incoercible diarrhoea.

At 2 months and 6 months, patients were contacted via mail or telephone calls. Notably, patients were also instructed to contact us via a dedicated mailbox if they report any AE out of our drown follow-up. A medical doctor conducted interviews to obtain information on the symptoms following the FMT. Individual interviews were conducted using open questions to capture their observations and the unique patients' symptom experiences, and then all items mentioned by the patients were summarised.

3.4 Long-term follow-up

Long-term follow-up in FMT treatment has only been evaluated in a few studies, with no report of harmful effects. From October 2020, all patients who had their first FMT at least 1 year before underwent a survey to record long-term AEs. Our survey ended in August 2022 and considered 43 patients treated from October 2020 to August 2021. All patients were contacted through mail or telephone calls by the Gastroenterologists of the Microbiota Study Group. New-onset symptoms and new-onset medical conditions that appeared after FMT were investigated and reordered. Patients were also asked about any new onset serious infections and life-threatening illnesses. Details of current bowel habits such as stool frequency, pain, discomfort, and consistency through the Bristol Stool Form Scale (BSFS) were recorded. Medical records were reassessed for patients who died after receiving FMT.

4. Results

4.1 Patients Characteristics

Between October 2020 and August 2022, 69 participants who underwent FMT treatment were enrolled and included in the study. Characteristics of patients completing the FMT survey are summarized in the following Table (Table 6).

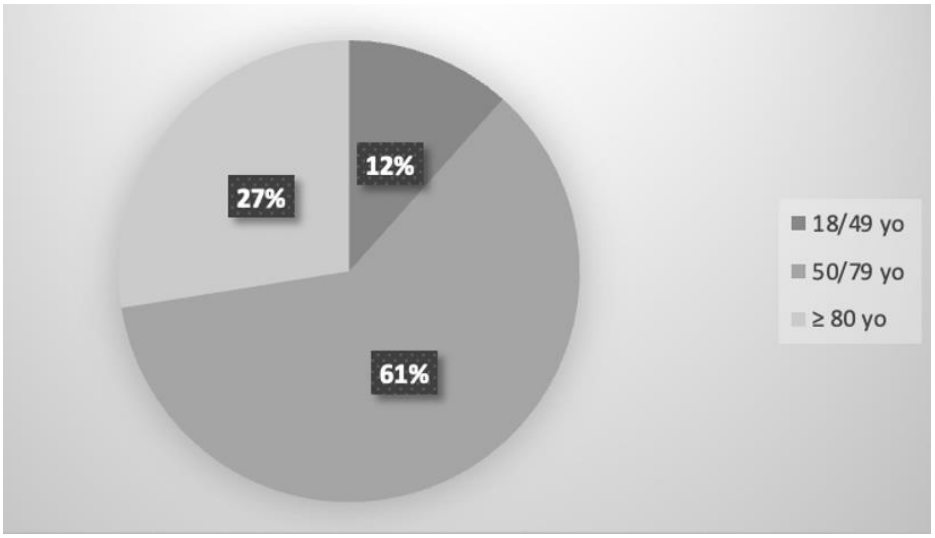
Patients had a mean age of around 68 years (range, 18–94). Recipients were almost equal, specifically 34 (49%) were female with a mean age of 67 years and 35 (51%) were male with a mean age of 69 years.

The most common gastrointestinal comorbidity at baseline was IBD, which was present in 19 (27,5%) patients; specifically 17 patients (24,6%) had ulcerative colitis (UC) and 2 patients (2,9%) had Crohn's disease (CD), other frequent comorbidities were the presence of diverticula in 6 patients (8,7%) and chronic gastritis in 5 patients (7,2%).

		N=69 (n %)
Sex		
	Male	35 (50,71%)
	Female	34 (49,27%)
Race		
	White	69 (100%)
Age		
	18/49 yo	8 (11,59%)
	50/79 yo	42 (60,86%)
	≥ 80 yo	19 (27,53%)

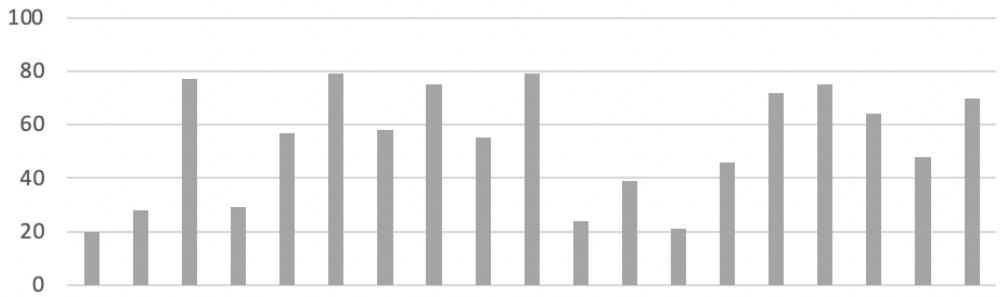
Table 6. Characteristics of recipient

During our follow-up, we divided into three groups patients for age: 18/49 years old (n=8; 11%); 50/79 years old (n=42; 60,9%) and, ≥ 80 years old (n=19; 27,5%) (Graph 2). As we know, CDI traditionally affects adults over the age of 65, perhaps due to a weakened immune system.



Graph 2. The age of patients included in the survey

As we reported, 19 patients with IBD were also included in the study. Traditionally, IBD has been described as having a bimodal incidence pattern, with the main peak being in the 15–25-year age group, and a second, smaller peak during the fifth to seventh decades. This being established, in our cohort group the average age of patients with IBD was 53 years (range 20/79 years old). Considering only 17 patients with UC, the average age was 51 years; the average age in CD patients was 75 years old (Graph 3).



Graph 3. The age of IBD patients included in the survey

At baseline, medical history and concomitant disorders were recorded through face-to-face pre-treatment visits. An accurate list of comorbidities has been drawn according to the last International Classification of Diseases (ICD-11) adopted by the 72nd World Health Assembly in 2019 and effective from 1st January 2022 (Table 7).

Our analysis has shown the most prevalent three comorbidities were ulcerative colitis (n=17; 24,6%); hypertension (n=17; 24,6%) and anaemia (n=13; 18,8%).

The focus on GI disease revealed the involvement of 39 patients (56,5%). The most common gastrointestinal conditions found were UC (n=17/69; 24,6%); presence of diverticula (n=6; 8,7%); chronic gastritis (n=5; 7,2%); Gastroesophageal reflux disease (n=3; 4,3%) and irritable bowel syndrome (IBS) (n=3; 4,3%).

We recognise the strong presence of cardiovascular disease in 34 patients (49,3%) include hypertension (n=17; 24,6%); arrhythmias (n=9; 13,04%); and valvular disease (n=9; 13%).

Gastrointestinal disease: 39 patients	
Crohn's disease (CD)	2/69 (2,89%)
Ulcerative colitis (UC)	17/69 (24,63%)
Diverticular disease	6/69 (8,69%)
Previous gastroparesis	1/69 (1,45%)
Lactose intolerance	1/69 (1,45%)
Chronic gastritis	5/69 (7,24%)
Gastroesophageal reflux disease	3/69 (4,34%)
Previous intestinal obstruction	1/69 (1,45%)
Previous volvulus	1/69 (1,45%)
Irritable bowel syndrome (IBS)	3/69 (4,34%)
Liver disease: 10 patients	
Cholestatic hepatitis	1/69 (1,45%)
Primary sclerosing cholangitis	1/69 (1,45%)
Cholelithiasis	3/69 (4,34%)
Primary biliary cholangitis	1/69 (1,45%)
Autoimmune liver diseases	1/69 (1,45%)
Previous ascites	3/69 (4,34%)
Cardiovascular disease: 34 patients	
Arrhythmia	9/69 (13,04%)
Cardiomyopathy	5/69 (7,24%)
Previous heart failure	9/69 (13,04%)
Hypertension	17/69 (24,63%)
Valvular disease	7/69 (10,14%)
Vascular disease	7/69 (10,14%)
Previous aortic dissection	3/69 (4,34%)
Neurological disease: 20 patients	
Dementia	5/69 (7,24%)
Parkinson's disease	3/69 (4,34%)
Peripheral neuropathy	3/69 (4,34%)
Previous stroke	5/69 (7,24%)
Multiple sclerosis	1/69 (1,45%)
Trigeminal neuralgia	2/69(2,89%)
Epilepsy	1/69 (1,45%)
Psychiatric disease: 6 patients	
Anxiety	2/69 (2,89%)
Depression	5/69 (7,24%)
Kidney disease: 20 patients	
Previous acute kidney injury	3/69 (4,34%)
Chronic kidney disease	9/69 (13,04%)
Kidney stone	9/69 (13,04%)
Pulmonary disease: 22 patients	
Asthma	2/69 (2,89%)
Bronchitis	1/69 (1,45%)
Previous pneumonia	3/69 (4,34%)
COPD	5/69 (7,24%)
Previous SarsCov2 infection	9/69 (13,04%)
Previous pulmonary embolism	5/69 (7,24%)
Haematological disease: 17 patients	
Anaemia	13/69 (18,84%)
Thrombotic thrombocytopenic purpura	1/69 (1,7%)
Myelodysplasia	5/69 (7,24%)
Old diagnosis of malignancies: 16 patients	
Spleen	1/69 (1,45%)
Breast	5/69 (7,24%)
Pancreas	1/69 (1,45%)
Endometrial	2/69 (2,89%)
Lymphoma	2/69 (2,89%)

	Prostate	3/69 (4,34%)
	Hypopharynx	1/69 (1,45%)
	Bones	2/69 (2,89%)
Metabolic and endocrine disorders: 25 patients		
	Diabetes Mellitus (DM)	9/69 (13,04%)
	Malnutrition	5/69 (7,24%)
	Hypovitaminosis	3/69 (4,34%)
	Thyropathy	10/69 (14,49%)
	Dyslipidemia	5/69 (7,24%)
	Parathyroid disease	1/69 (1,45%)
Urogenital disease: 2 patients		
	Prostatic hypertrophy	2/69 (2,89%)
Dermatological disease: 5 patients		
	Dermatitis	5/69 (7,24%)
Immunologic disorders: 6 patients		
	Nickel allergy	1/69 (1,45%)
	Sjogren's syndrome	1/69 (1,45%)
	Multiple allergies	5/69 (7,24%)
Previous Surgery: 28 patients		
	Adenoidectomy	1/69 (1,45%)
	Hernioplasty	3/69 (4,34%)
	Cholecystectomy	5/69 (7,24%)
	Gastrectomy	1/69 (1,45%)
	Appendicectomy	7/69 (10,14%)
	Bowel resection	3/69 (4,34%)
	Heart transplant	1/69 (1,45%)
	Varicocele	1/69 (1,45%)
	Prostatectomy	3/69 (4,34%)
	Thyroidectomy	5/69 (7,24%)
	Hysterectomy	3/69 (4,34%)
Rheumatic disease: 8 patients		
	Arthrosis	8/69 (11,59%)
	Muscular dystrophies	1/69 (1,45%)
Previous infections: 6 patients		
	Proteus	1/69 (1,45%)
	Klebsiella oxytoca	1/69 (1,45%)
	E. coli ESBL+	1/69 (1,45%)
	Enthamaeba Histolytica	1/69 (1,45%)
	E. coli CTX-M-	1/69 (1,45%)
	A. baumanii	1/69 (1,45%)

Table 7. Recorded patient comorbidities (Reference I and II available in supplementary material)

The presence of comorbidities was analysed and used to calculate the Charlson comorbidity index (CCI). The mean CCI in these patients resulted in 5 (weighted) as shown in the following Table (Table 8).

Charlson Comorbidity Index (CCI)	
Score	N patients (%)
0	4 (5,79%)
1	12 (17,39%)
2	7 (10,14)
3	4 (5,79%)
4	8 (11,59%)
5	6 (8,69%)
6	10 (14,49%)
7	4 (5,79%)
8	4 (5,79%)
9	3 (4,34%)
10	4 (5,79%)
11	1 (1,44%)
12	2 (2,89%)
10- years of survival	
%	N Patients (%)
0-25%	18 (26,08%)
26-50%	21 (30,43%)
51-75%	22 (31,88%)
76-100%	8 (11,59%)

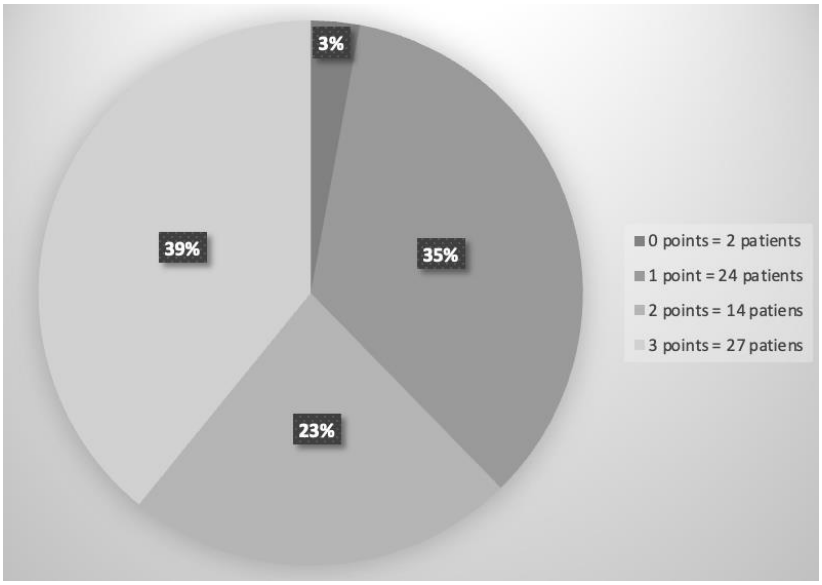
Table 8. Charlson comorbidity index of patients and 10-years survival

4.2 Faecal Microbiota Transplantation: details of infusions

All participants (n=69) received a faecal microbiota transplant from an unrelated donor that undergoing a meticulous selection process (90). All procedures were performed by colonoscopy using standard or paediatric colonoscopes and carbon dioxide insufflation, under sedation. Despite each patient underwent bowel cleansing with 4 L of macrogol (SELG ESSE) the day before the procedure, not every patient has achieved satisfactory bowel preparation. As reported by the figure below (Graph 4), we assigned:

- 0 point = Unprepared colon segment with mucosa not seen due to solid stool that cannot be cleared
- 1 point= Portion of the mucosa of the colon segment seen, but other areas of the colon segment not well seen due to staining, residual stool and/or opaque liquid.
- 2 points = Minor amount of residual staining, small fragments of stool and/or opaque liquid, but mucosa of colon segment seen well.
- 3 points = Entire mucosa of colon segment seen well with no residual staining, small fragments of stool or opaque liquid. The wording of the scale was finalized after incorporating feedback from three colleagues experienced in colonoscopy.

2 patients (2,9%) presented an unprepared segment of the colon with areas of non-visible mucosa due to the presence of solid stools that cannot be eliminated with the aid of the colonoscope. 24 patients (34,8%) had areas of visible colonic mucosa, but other areas not clearly visible due to residual stool. 16 patients (23,2%) presented small fragments of liquid stool, but the mucous membrane of the colon segment could be seen. In 27 patients (39,2%), the entire colonic mucosa was clearly visible without fragments of faeces.



Graph 4. Boston Bowel Preparation Scale (BBPS) calculated in recipients.

Details of the procedure were recorded and reported in Table 9. All procedures were performed by four expert endoscopists (G.C., G.I., S.B., and F.S.). All faecal infusate samples were prepared in the microbiology laboratory of our hospital, using at least 50 grams of frozen faeces for each sample stored at −80°C. Faecal infusates were delivered 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. The mean volume administered was 250 mL (range, 200 - 280 mL). During the insertion and removal of the colonoscope, operators were able to assess the presence of pseudomembranes, presence of polyps and other inflammatory signs of the large bowel. After the procedures, patients were monitored in the recovery room of the endoscopy centre for 2 - 3 hours after the procedure.

		N=69 (n %)
Donor type		
	Unrelated donor	69 (100%)
Patient care (pre-FMT)		
	Outpatient	36 (52,17%)
	Hospitalized	33 (47,82%)
Patient care (post-FMT)		
	Hospital	38 (55,07%)
	Home	31 (44,92%)
Route of delivery		
	Colonoscopy	69 (100%)
Material inflated		
	Frozen	69 (100%)
Volume of faces administered (tot)		
	200 mL	6 (8,69%)
	240 mL	15 (21,73%)
	250 mL	55 (79,71%)
	280 mL	1 (1,44%)
Pseudomembranes		
	Presence	12 (17,39%)
	Absence	57 (82,60%)

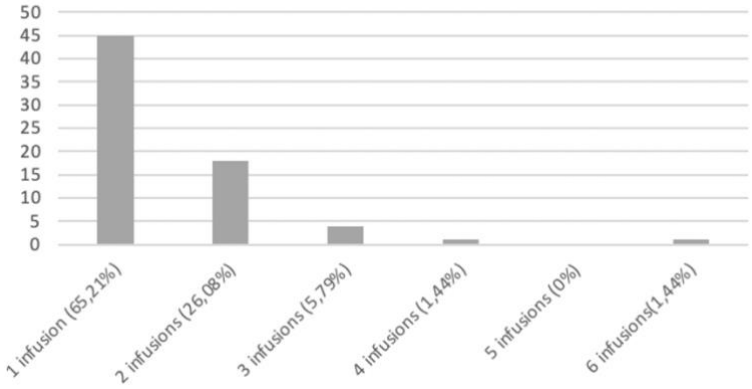
Table 9. Details of procedure

Single-infusion FMT (SIF) has been commonly accepted as a satisfactory option for the treatment of rCDI; recently, multiple infusion FMT (MIF) is demonstrating even higher cure rates than SIF (67, 87).

In our cohort, 45 patients received a single infusion (65,2%) and 24 patients received multiple infusions (34,8%) (graph number of infusions). Specifically, 18 patients received a second infusion (26%), 4 patients received a third infusion (5,8%), one patient received a fourth infusion (1,44%), and another patient received a sixth infusion (1,44%). Almost all studies in CDI and clinical guidelines recognize that sequential FMT is higher effective than a single infusion, at least in specific situations such

as such as severe CDI or enema infusion (91). None procedural complications were observed.

The data in the literature report that multiple infusions have increased the efficacy rates of FMT, this seems to be also linked to the use of low dosages of faecal material (about 50 g). Both of these findings underscore the importance of providing enough biomass to restore a healthy microbiota, either by infusing large amounts of stool at once or by repeating infusions (66).

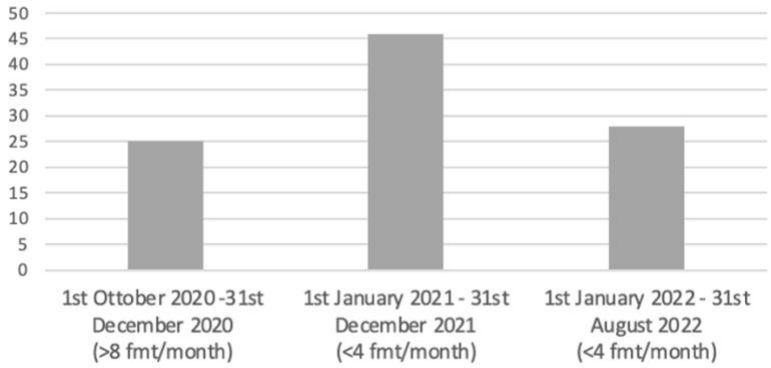


Graph 5. Number of infusions

Following the outbreak of SARS-CoV-2 infection, health facilities have significantly reduced elective activities both to avoid the potential transmission of the virus and to shift human and structural resources to the management of the infection. Noteworthy, CDI continues to have high morbidity and mortality rate therefore FMT has been considered among the non-postponable gastroenterological procedures during the COVID-19 pandemic, particularly in high-risk patients in whom FMT could be lifesaving (92).

However, the pandemic has raised several issues concerning donor recruitment, availability of faecal material, new screening protocols, and reorganization of healthcare staff. Even our centre has seen a sharp decline

in the number of procedures performed. In the following graph, only the number of FMT performed in patients with CDI is considered. In 2020, about 8 FMT were performed per month, this is linked to the fact that the faecal material used dates back to an enrollment of patients before the covid19 emergency. As the graph shows, in 2021 and 2022 the number of infusions performed per month has been halved (Graph 6).



Graph 6. Number of infusions performed

4.3 Short-term follow-up

Data recorded from the scientific literature recognise minor adverse events post-FMT, often transient, such as abdominal discomfort, alterations in weight, and low-grade fever (82). More rarely, serious side effects are related to possible complications of the procedure. According to the data, the two most seen side effects after FMT are bloating and diarrhoea in the first 24 hours. In a recent systematic review, FMT-related adverse events were summarised by collecting data for over 20 years. Essentially, 85 unique types of AEs were reported in 24% of FMT procedures (1347/5688) during or after FMT, including 6% (246/4241) of patients with SAEs (73).

Our surveillance based the follow-up at 2 weeks through face-to-face outpatients' visits at the Fondazione Policlinico Universitario "A. Gemelli" and, at 2 months and 6 months, patients were contacted via mail or telephone calls.

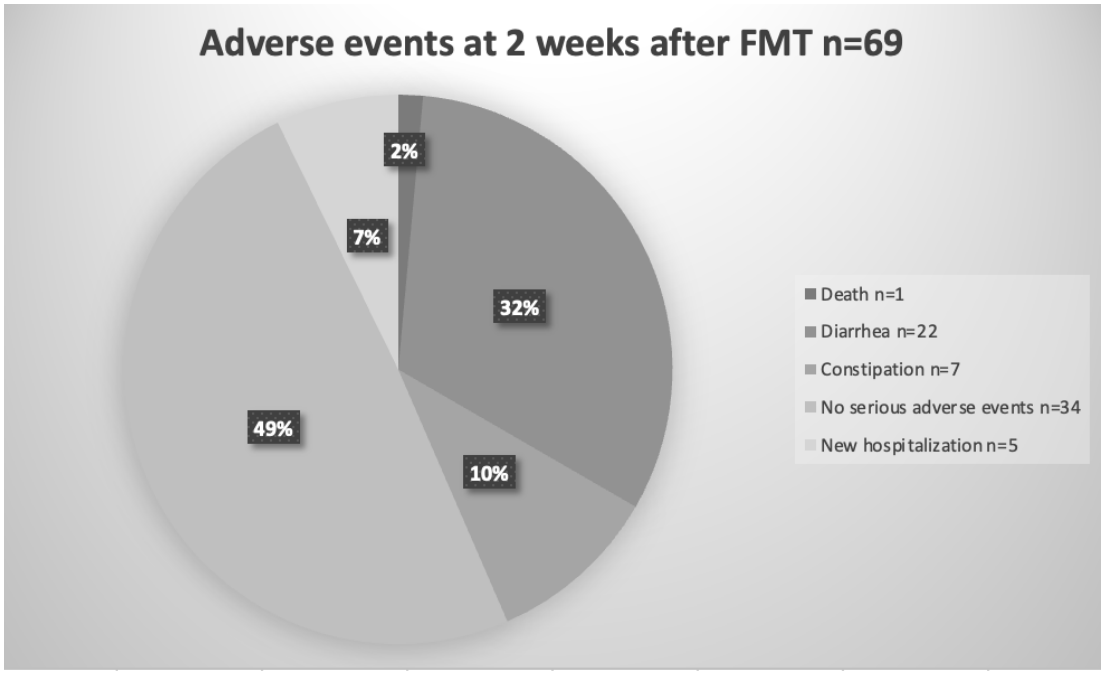
At 2 weeks, the survey response rate was 100% (n=69); 91,3% at 2 months (n=63); and 82,6% at 6 months (n=57) (Table 10).

SHORT TERM FOLLOW-UP = Patients undergoing FMT from October 2020 to August 2022 (n=69)		
Follow-up at 2 weeks post-FMT	Responded to survey: n=68 (98,55%)	New death n=1 (1,44%)
Follow-up at 2 months post-FMT	Responded to survey: n=63 (91,30%)	New death n=2 (2,44%) No surveillance n=3 (4,34%)
Follow-up at 6 months post-FMT	Responded to survey: n=57 (82,60%)	New death n=3 (4,34%) No surveillance n=6 (6,69%)

Table 10. Short-term Follow-up

4.3.1 Two weeks follow-up

At 2 weeks follow-up post-FMT, patients experienced mild symptoms such as abdominal pain, bloating, nausea, low-grade fever, and constipation, which are often self-limiting within a few hours or days (Graph 7). Less than a third of patients reported having diarrhoea at 2 weeks (n=22; 33%), although more than half of them reported diarrhoea that lasted less than a week. In the case of unformed stools, the stool culture test is not recommended but is considered if 3 or more diarrhoea stools occur per day after a few weeks. It is crucial to note that the polymerase chain reaction test for *C. difficile* toxin can remain positive for 30 days after successful treatment, which is another reason not to test asymptomatic patients undergoing FMT. Constipation was seen transiently 2 weeks after the procedure (n=7; 10%) but usually resolves within 1-2 weeks. There were 5 (7%) new hospitalizations within 2 weeks of FMT. There were no infectious complications directly attributable to FMT. Two hospitalizations were linked to bone fractures, clearly unrelated to FMT. Two hospitalizations were linked to heart failure and, one catheter line infection requiring hospitalization occurred and was probably unrelated to FMT. A patient (1,44%) with severe heart disease died two weeks after FMT from cardiological complications, despite having achieved resolution of diarrhoea and gastrointestinal symptoms.

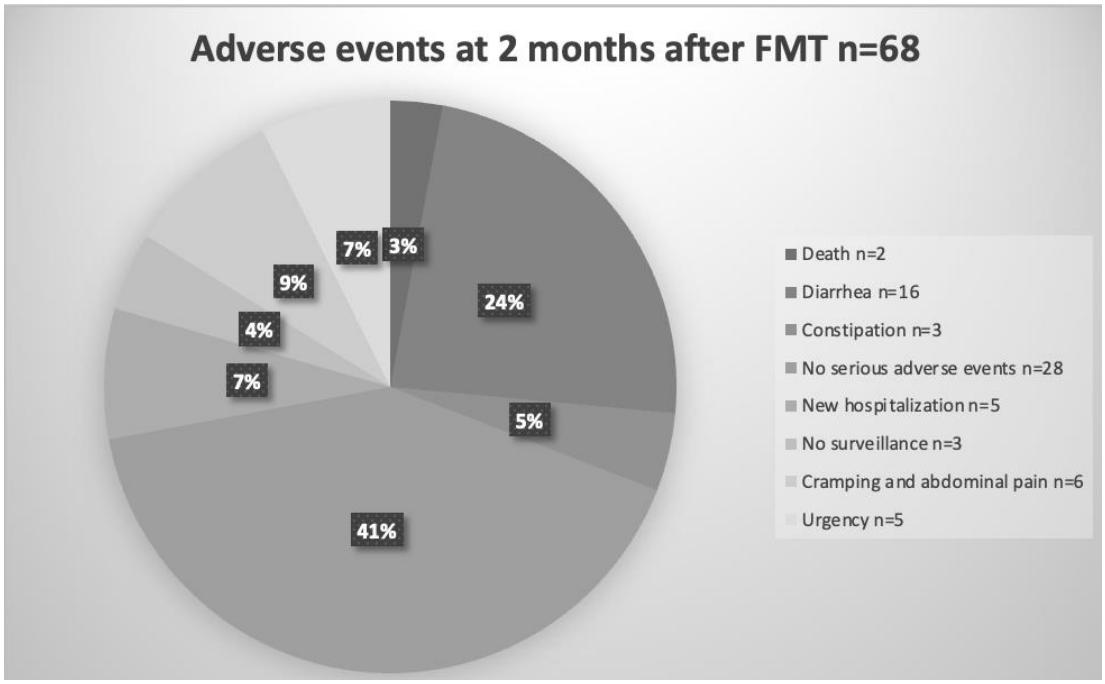


Graph 7. Adverse events registered at 2 weeks

4.3.2 Two months follow-up

In the two months following the FMT, 16 (23,5%) patients experienced episodic diarrhoea and 3 patients (4,4%) had constipation, 6 patients (8,8%) reported short periods of bloating and abdominal cramps (Graph 8). In all patients, these symptoms resolved within days or weeks. There were 5 (7,3%) new hospitalizations within 2 months of FMT. This was attributed to the fact that 4 of these 5 patients reported resolution of symptoms associated with CDI and were able to undergo surgery that was delayed due to infection (drainage of recurrent parotid abscesses; Transcatheter aortic valve replacement (TAVR); Atrial fibrillation ablation and, Hip replacement). There was no connection directly attributable to FMT. On the other hand, a new hospitalization was recorded in a patient who had suffered from UC since the age of 23 years. He later developed severe UC that was resistant to medical treatment, and CDI so 2 months after FMT a restorative proctocolectomy with ileal pouch-anal anastomosis (RPC-IPAA) was performed.

After 2 months of follow-up, 3 patients were lost to follow-up, and 2 had decreased, the cause was associated with pre-existing chronic progressive illnesses. A 79 patient with a known history of chronic kidney disease (CKD) died of end-stage renal failure (ESRD) after two months from the FMT; another patient who was hospitalized in the intensive care unit died of myocardial infarction after 43 days from the FMT.



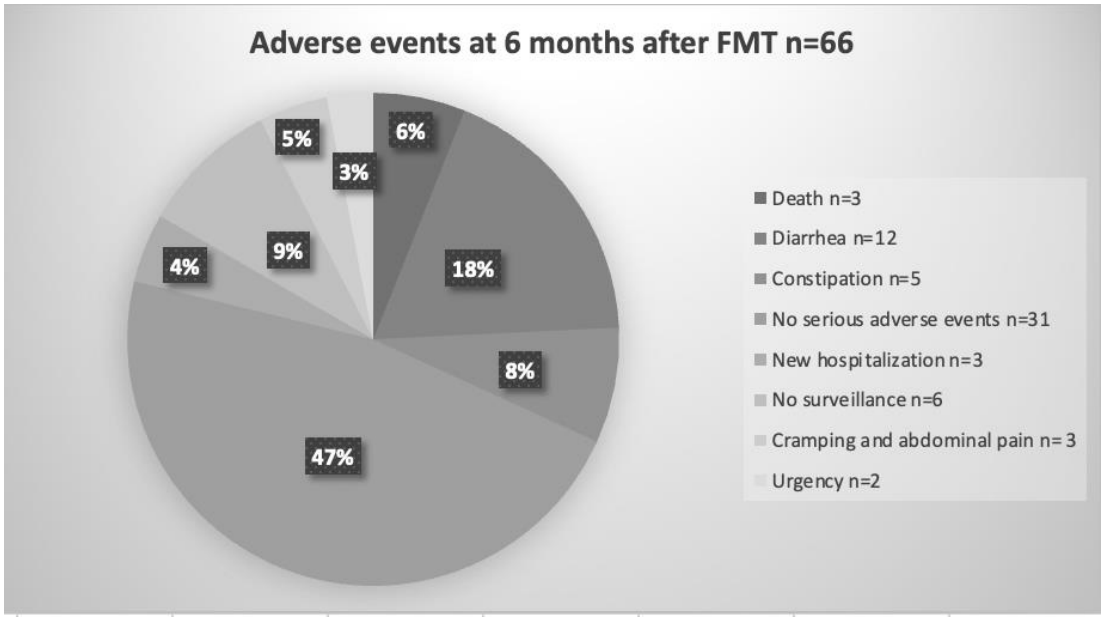
Graph 8. Adverse events registered at 2 months

As we established, treatment success was defined as clinical cure 8 weeks after FMT with the absence of diarrhoea (excluding isolated episodes of diarrhoea) and with no recurrence. Excluding 3 patients lost at 2 months follow up, we analysed the cure rate in 66 patients. 40 patients (60,6%) were cured after only one infusion; 17 additional patients (25,75%) were cured after sequential infusions (from 2 to 6 infusions, Graph 5), with an overall cure rate around 86,36%. No significant differences were found between male and female patients. 7 patients (10,6%) experienced an early (≤8 weeks post-FMT) CDI recurrence. In three (3,5%) of these, CDI recurrence was associated with the use of antibiotics within 8 weeks after FMT. In the other four patients (6%), no antibiotics had been prescribed post-FMT.

4.3.3. Six months follow-up

At 6 months follow-up post-FMT, patients experienced mild symptoms such as diarrhoea, abdominal pain, weight gain, and constipation, which are often self-limiting within a few days or weeks (Graph 7). After 6 months from the FMT, 6 patients were lost to follow-up (9%), of which 4 patients were found to be untraceable and 2 refused to participate in surveillance. In the remaining patients, 17 (25,75%) showed changes in bowel habits in the last 6 months: 12 patients (18,2%) reported multiple episodes of diarrhoea and 5 (7,5%) reported transiently constipation. 8 patients performed the stool culture test and *C. difficile* toxin, without any doctor's recommendation, which resulted negative. There were 3 (4,5%) new hospitalizations within 6 months after FMT. It is noteworthy that none of those hospitalizations was linked to FMT. One UC patient underwent a colectomy 5 months after FMT for progressive worsening of UC, which had not improved after CDI treatment. Another patient was admitted to the hospital for a cerebrovascular accident; the third patient suffered a fracture of the left femur that required surgery.

Three deaths (4,5%) were reported at 6 months follow-up, with a median age of 81 years old. All occurred more than 4 months after FMT and were related to chronic progressive illnesses unrelated to CDI. Specifically, one patient died of heart failure 4 months after FMT, another patient died of ischemic stroke 5 months after FMT, and the third patient died 6 months after FMT of chronic kidney disease.



Graph 9. Adverse events registered at 6 months

4.4 Long-term follow-up

For long-term follow-up, the time point was assessed at 1-year after FMT. Our survey ended in August 2022 therefore the patients should have been treated from October 2020 to August 2021. According to our eligible criteria, only 43 patients (62,3%) were enrolled in our long-term follow-up. All patients were contacted through telephone calls by the Gastroenterologists of the Microbiota Study Group. New-onset symptoms and new-onset medical conditions that appeared after FMT were investigated and recorded. Patients were also asked about any new onset serious infections and life-threatening illnesses. Details of current bowel habits such as stool frequency, pain, and discomfort were recorded. Medical records were reassessed for patients who died after receiving FMT.

LONG TERM FOLLOW-UP = Patients undergoing FMT from October 2020 to August 2021 (n=43)		
Follow-up at 1-year post-FMT →	Responded to survey: n=35 (81,4%) →	New death n=1 (2,325%) No surveillance n=7 (16,3%) Deaths in the previous follow-up n=6

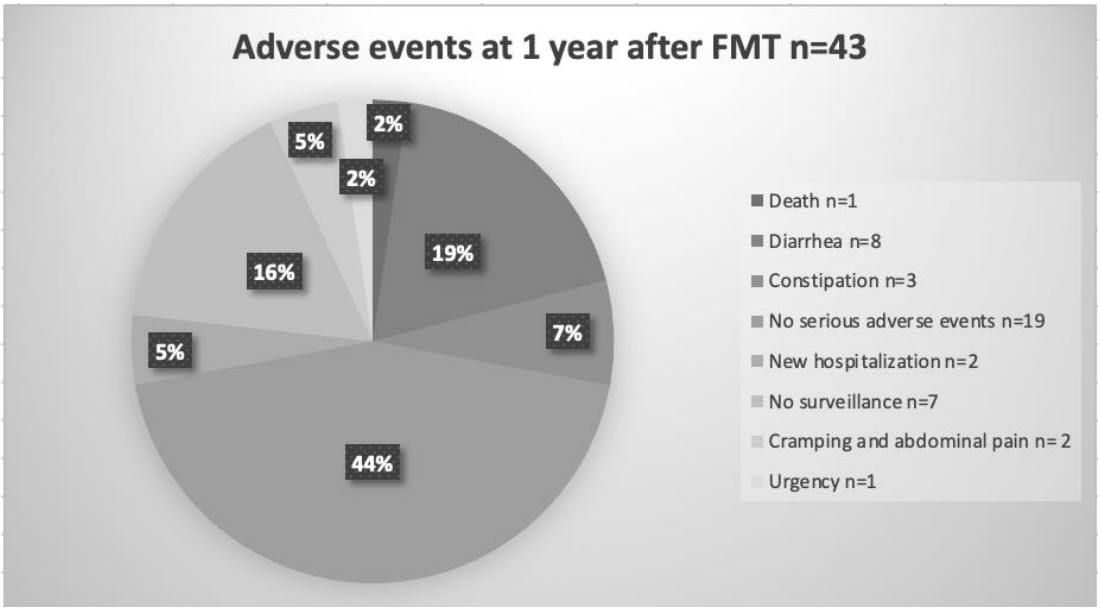
Table 11. Long-term Follow-up

4.4.1 One-year follow-up

A total of 43 patients were included to assess the follow-up within 1 years after FMT. The median age at that timepoint was 68 years old (ranging 20–90 years). Loss of follow-up was noted in 7 (16,3%) patients who were also among the oldest, rates did not differ significantly between females and males.

Apart from intermittent mild abdominal pain (4,65%), episodes of diarrhoea (18,65%) and constipation (7%) that occurred sporadically over the year after FMT, no adverse events were reported during the follow-up period. It is noteworthy that 2 patients (4,65%) developed diverticulitis at 7 and 10 months after FMT which required hospitalization and subsequent surgery in both patients.

To our knowledge, only one patient died between 6 and 12 months after FMT. A 79-year-old man (2.32%) with a previous history of recurrent urinary tract infections (UTIs) developed sepsis 9 months after FMT for which he received antibiotics without developing subsequent CDI. After a few months, the patient died.



Graph 10. Adverse events registered at 1-year

5. Discussion

In this single-center cohort study, we report the short-term and long-term safety, from 2 weeks to 1 year, after FMT. The short-term safety of FMT in patients with rCDI is thoroughly established from the increasing number of patients treated worldwide. As a general rule, short-term SAEs after FMT are rare and mostly procedure related. Our results, in combination with a review of the available literature, suggest that FMT does not cause serious short-term adverse events. The main symptoms recorded immediately after the procedure were nausea, low fever, and diarrhoea. All manifestations were self-limiting and the patients usually experienced resolution of symptoms after 24-72 hours.

During short-term follow-up, diarrhoea was reported approximately in up to a third of patients at two weeks after FMT. Although, episodes of diarrhoea lasted less than a week, with resolution in more than half patients. In the subsequent surveillance, the recorded episodes were lower except for patients with IBD in which there were alterations of the bowel habits related to the clinic of the individual patients. Abdominal pain, or cramps, bloating, flatulence, and constipation were self-limiting in all analyzed patients along all the surveillance steps.

A special mention should be made for patients with CDI and underlying IBD, for whom infection with *C.difficile* represents a demanding challenge. IBD patients have been shown to develop CDI three times more frequently than the general population and have an estimated 10% chance of contracting *C.difficile* (93, 94). Interestingly, about half of the IBD patients had complications in our follow up period and three of which were treated with steroids and one patient required a colectomy. Colon disease activity in these patients at the time of FMT ranged from mild to severe. However, they are all known to have severe (and often refractory) disease

that led to FMT, and despite the short-term complications, most patients benefited from FMT in terms of CDI and the course of their IBD. It is not possible to determine whether these exacerbations were attributable to FMT, CDI, or progression of the underlying disease. It is emphasized that our surveillance has the main purpose of recording adverse effects in patients with CDI treated with FMT for which it was not considered necessary to distinguish the subgroups.

For long-term follow-up, the time point was assessed at 1-year after FMT. Our survey ended in August 2022 therefore the patients should have been treated from October 2020 to August 2021. According to our eligible criteria, only 43 patients (62,3%) were enrolled in our long-term follow-up. Weaknesses of this study are inherent to the design and included missing or incomplete data of part of the patients treated. 6 out of 69 (8,7%) patients were lost to 6 months follow-up, and 7 out of 43 (16,3%) patients were lost to 1 year follow-up.

At the end of the follow-up, 7 patients had died; none of the deaths was directly attributable to FMT, as evaluated by the experts, and probably reflects the frailty of patients with rCDI, advanced age (mean 68 years), comorbidities and a low CCS. The mortality rate among the patients included in the study agrees with other studies (95, 96). There was no indication that FMT ameliorated or deteriorated the clinical course of these pre-existing disorders. Therefore, physicians may be reluctant to prescribe FMT to older or weaker patients assuming they are "at high risk". Indeed, it has been shown that patients with relapsed CDI have marked presence in the GM of sample of the Enterobacteriaceae family of Proteobacteriaceae (97, 98), which are often relatively invasive pathogens that may be responsible for other infectious complications in these patients. On the other hand, FMT leads to the restoration of the dominance

of Bacteroidetes and Firmicutes in the distal gut microbiota. It is reasonable to expect that normalized intestinal microbial ecology should improve colonization resistance to potential pathogens in older and immunocompromised patients.

Moreover, our surveillance was born in a coexistence scenario with COVID-19, which severely tested the health systems of individual countries around the world. Therefore, we have learned to rationalize health services with criteria dictated in the first place from the emergency and then from the reasoned planning, with variations in degrees of difficulty in different clinical scenarios. In this context, FMT finds its place as a life-saving procedure for a considerable number of patients with CDI in clinical practice. Solutions had to be found to maintain donor recruitment standards and to allow virtual follow-up visits (especially post-treatment ones) to be carried out safely rather than in person. In addition, when possible, attempts have been made to limit the use of multi-donors to limit the risk of transmission of COVID-19. As suggested for clinical practice, the use of frozen stool was preferred to fresh material, although SARS-CoV-2 can likely survive the storage conditions. Manipulation of faeces aliquots and storage of stool samples before and after FMT was carried out in a highly safe environment.

Further implementation of FMT is hampered by the lack of uniform guidelines, concerns about safety, and remaining uncertainty about long-term side effects. Our results were comparable to other studies and showed a success rate around 86,36% in 69 patients, with a primary cure rate of this study supports the currently available evidence that FMT is a very effective treatment for recurrent CDI.

7. Conclusion

Enormous progress has been made in the study of FMT for the management of CDI over the past decade. There are countless ongoing studies evaluating FMT as a potential therapy for other conditions of both gastrointestinal and extra-intestinal diseases. However, there is still much we do not understand about the underlying mechanism of the microbiome and its role in the physiology and treatment of the disease. Clinicians and patients alike will benefit from a better understanding of the risks of FMT and clear outlined protocols to assess short- and long-term adverse events (95). There is a need for other high, prominent, prospective, long-term follow-up randomised controlled clinical trials investigating selected donors and recipients to assess this promising therapy's long-term safety and benefit-risk profile.

The enormous impact of COVID-19 on many facets of research has already been recognized. Mandatory strict screening guidelines for donors are the necessity of the moment, although screening cannot prevent unexpected infections. The COVID-19 pandemic challenged health systems globally, and it is assumed that it will also be present shortly, forcing us to adjust overall clinical-procedural standards. Finally, the development of experimental microbiome therapies with defined microbial consortia will provide greater confidence in drug purity, identity and potency, and risk mitigation for improved patient safety in terms of both short and long term (78).

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9. Supplementary material

History of Sars Cov-2 infection	Gastrointestinal disease	Hepatopathy	Cardiovascular disease	Neurological disease	Psychiatric disorders	Kidney disease	Polmunary disease	Haematological disorders	Malignancies	Metabolic and endocrine disorders	Urogenital disease	Dematological disease	Immunoallergic disorders	Surgery	Rheumatic disease	OTHER GI Infections
0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0
1	1	1	0	1	0	0	1	0	0	0	0	1	1	0	0	0
0	1	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	1	1	0	1	0	0	0	0	1	0	0	0	1	1
0	1	0	1	1	0	1	1	1	0	0	1	0	0	0	0	1
0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0
0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
0	1	1	1	0	0	0	1	0	0	0	0	0	1	1	0	0
0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
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0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0
0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Reference I of Table 7.

History of Sars Cov-2 infection	Gastrointestinal disease	Hepatopathy	Cardiovascular disease	Neurological disease	Psychiatric disorders	Kidney disease	Polmunary disease	Haematological disorders	Malignancies	Metabolic and endocrine disorders	Urogenital disease	Dermatological disease	Immunoallergic disorders	Surgery	Rheumatic disease	OTHER GI Infections
0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	1	1	0	1	1	0	1	1	0	0	0	1	0	0
1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1
0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	1	1	1	1	1	0	0	0	1	0	1
0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0
0	0	1	1	0	1	0	1	0	0	1	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
0	0	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0
1	0	0	1	1	0	1	1	0	1	1	0	0	0	0	0	1
0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	1	0	0	0	0	1	1	0	0	0	1	1	0
0	0	0	1	0	1	1	1	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	1	1	0	1	0	0	1	1	0	0	0
0	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0
0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0
0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0
0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0
0	1	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0
0	1	0	1	0	1	0	1	0	0	1	0	0	1	1	0	0
1	1	1	1	1	1	0	1	1	0	1	1	0	0	1	0	0
1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0
0	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
0	0	0	1	0	0	0	0	1	0	1	0	0	1	1	1	1

Reference II of Table 7.

10. Acknowledgments

On this research trip, I came across numerous people who helped me and traveled with me. Therefore, for my success I should express my sense of gratitude to those people who have unconditionally supported, helped, and encouraged me in multiple situations.

I would like to express my deep gratitude to my main tutor, Professor Giovanni Raimondo, who welcomed me on this long path and believed in my ability to carry it out. I am very grateful to him for his lifelong support and scientific guidance.

I also thank Professor Walter Fries and his Team of Clinical Unit of Gastroenterology and Chronic Bowel Disorders of Messina, for giving me wise advice and indicating me the route. The guidance and encouragement have been extremely valuable both for the work of this path and for my professional development.

I wish to sincerely thank Professor Giovanni Cammarota as my primary supervisor, mentor, and guide during my Research Period at Fondazione Policlinico A Gemelli IRCCS, Catholic University of Medicine in Rome. More than assisting me with the practical, technical, and theoretical aspects of my research, which has been instrumental, hi encourages me, challenges me, provides opportunities, promotes me and my work, and has fostered enormous growth in me as an early career academic.

I am also very glad and thankful for having met and worked with Dr Gianluca Ianiro for his willing collaboration, offering me insightful comments and suggestions. I deeply appreciate his careful revision of all my work. I would also like to thank him for making me feel part of the Team.

I deeply appreciate and thank the collaboration of my colleague and friend Dr Serena Porcari for her availability and kindness, assistance at every stage, patience in teaching me and constantly encourage me.

I thank my wonderful parents for their unconditional love and support. They are the pillar of my life, the basis of my days. Thank you for always supporting me steadfastly, for your precious advice and your ability to listen to me, for always being by my side.