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FECAL MICROBIOTA TRANSPLANTATION FOR RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION: EFFICACY AND LONG-TERM SAFETY

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ŽARNYNO MIKROBIOTOS TRANSPLANTACIJA PASIKARTOJANČIOS *CLOSTRIDIOIDES DIFFICILE* INFEKCIJOS GYDYMUI: EFEKTYVUMAS IR ILGALAIKIS SAUGUMAS

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CONTENTS

ABBREVIATIONS

CDI	–	*Clostridioides difficile* infection
rCDI	–	recurrent *Clostridioides difficile* infection
C. difficile	–	*Clostridioides difficile*
HAI	–	hospital-acquired infections
FMT	–	fecal microbiome transplantation
GI	–	gastrointestinal
NAAT	–	Nucleic Acid Amplification Testing
GDH	–	glutamate dehydrogenase
EIA	–	enzyme immunoassay
BEZ	–	Bezlotoxumab
SAE	–	serious adverse event
AGA	–	the American Gastroenterological Association
CD	–	Crohn's disease
STEC	–	Shiga toxin-producing *E. coli*
EPEC	–	enteropathogenic *E. coli*
FDA	–	Food and Drug Administration
LBPs	–	live biotherapeutic products
UC	–	ulcerative colitis
RCT	–	randomized controlled trial
ARB	–	antibiotic-resistant bacteria
CRP	–	C-reactive protein
ELISA	–	Enzyme-Linked Immunosorbent Assay
HIV-1	–	Human Immunodeficiency Virus, type 1
HIV-2	–	Human Immunodeficiency Virus, type 2
EBV	–	Epstein-Barr virus
CMV	–	cytomegalovirus
ESBL	–	extended-spectrum beta-lactamase
CRE	–	carbapenem-resistant *Enterobacteriaceae*
VRE	–	vancomycin-resistant enterococci
MRSA	–	methicillin-resistant *Staphylococcus aureus*
GP	–	general practitioners
MDRO	–	multidrug-resistant organism

INTRODUCTION

In recent years, the concept of a healthy gut has become inseparable from the recognition of the gut microbiome. Although microorganisms colonize various regions of the human body, the highest density is found in the gastrointestinal tract [1]. It is now well established that these microorganisms play essential roles in food digestion, nutrient absorption, energy and vitamin production, modulation of gut inflammation, and regulation of immune system function [2].

The composition of the human gut microbiota is influenced by both internal (host-related) and external (environmental) factors. Key internal determinants include gastric pH, bile acid secretion, intestinal motility, and immune activity. External or extrinsic factors such as diet composition, hygiene practices, chemical exposures, environmental pollution, physical activity, stress levels, and sleep patterns also significantly contribute to shaping the microbiome [1, 3].

The foundational composition of the human microbiota is typically established during the first 3 to 4 years of life [4]. While the gut microbiome is generally stable and capable of returning to its baseline state after short-term disruptions, it remains vulnerable to chronic alterations. These may be induced by long-term exposure to Western dietary patterns, food additives, environmental toxins, and especially by the widespread use of antibiotics [5]. Broad-spectrum antibiotics cause prolonged disruptions in the composition of the gut microbiome, a condition commonly referred to as dysbiosis [6]. Recovery of the microbiota following antibiotic exposure is often slow and, in some cases, incomplete. This altered microbial environment provides favorable conditions for the overgrowth of pathogenic bacteria, thereby increasing the risk of antibiotic-associated diarrhea [7, 8].

Between 2001 and 2012, the annual incidence of *Clostridioides difficile* infection (CDI) increased by 43%, while cases of multiple recurrent CDI (rCDI) rose by 188% during the same period according to data in United states of America [9]. Data from a laboratory-based surveillance study conducted in 2011 estimated the annual incidence of CDI in the United States to be approximately 453,000 cases, with 14,000 deaths directly attributable to this infection [10]. Surveillance data from the European Centre for Disease Prevention and Control during 2016–2017 reported that *Clostridioides difficile* (*C. difficile*) accounted for 4.9% of all hospital-acquired infections (HAIs) and 54.6% of hospital-acquired gastrointestinal infections across Europe [11]. In this analysis, *C. difficile* was responsible for 8.9% of HAIs reported in Lithuania, with an increase to 21.2% during the 2022–2023 period

[12]. However, comparing CDI epidemiology across different years, regions, and countries remains challenging due to varying diagnostic approaches, case definitions, and reporting standards. A meta-analysis of studies from 2009 to 2019 demonstrated heterogeneous results across Europe, with the highest HAI CDI incidence reported in Poland at 6.18 cases per 10,000 patient-days, and the lowest in the United Kingdom at 1.99 cases per 10,000 patient-days [13]. Despite inconsistent and fragmented epidemiological data, the growing threat of hospital-associated infections such as CDI remains undeniable.

As CDI has become an increasing burden on healthcare systems and more data has been gathered, researchers have identified major risk factors for CDI. These include contact with healthcare facilities, older age (> 65 years), and antibiotic use for other infections [14]. Broad-spectrum antibiotics are widely used in the current healthcare environment and are major risk factors for gut dysbiosis. Disruption of the intestinal microbiome creates favorable conditions for CDI, as it can take months for microbiome diversity to return to its original state [15, 16]. The most important clinical sign of infection is diarrhea (≥ 3 unformed stools in 24 hours) accompanied by positive *C. difficile* toxin tests, as recommended by clinical guidelines [14, 17, 18].

The first episodes of CDI are treated with oral antibiotics, but recurrent disease requires the restoration of the altered gut microbiota. Over the past decade, fecal microbiota transplantation (FMT) has emerged as the preferred treatment modality for rCDI. In 2013 van Nood et al. [19] conducted the first randomized, controlled clinical trial evaluating the efficacy of FMT for the treatment of rCDI. An infusion of fecal material via a nasoenteric tube after treatment with oral vancomycin was found to be superior to vancomycin therapy alone. Later studies explored different FMT modalities – colonoscopy, enemas, oral capsules with promising results in treating rCDI [14, 18, 20–23]. Despite the initial success of FMT, large-scale studies are needed to evaluate its real-world efficacy across different FMT modalities [19, 24–32]. As highlighted in the international FMT consensus, challenges such as a lack of expert centers, difficulties in donor recruitment, regulatory hurdles, and significant safety concerns persists [21].

Recurrent and refractory *C. difficile* colitis poses significant clinical challenges, as treatment with antimicrobial agents provides only short-term resolution and fails to achieve long-term efficacy. It is well established that the risk of recurrent disease after the first episode of CDI is approximately 20%, increasing to a staggering 60% after multiple recurrences [33, 34]. Despite being a guideline-recommended curative therapy for recurrent and refractory infection, FMT remains a state-of-the-art treatment method [14, 21–23, 35]. However, access to FMT is limited for many CDI patients, as

establishing a functional FMT center requires significant expertise and effort [36]. While the number of FMT procedures performed continues to grow, additional studies are needed to evaluate the efficacy of different FMT methods, as well as their short- and long-term safety.

Scientific novelty

In current clinical practice, the majority of reported CDI treatments with FMT are delivered via the lower gastrointestinal (GI) route, most commonly by infusing fecal material during colonoscopy [37]. This research investigates the **therapeutic efficacy of FMT when fecal material is introduced via the upper GI tract** using two different methods – **enteric tube and oral capsules** – offering an alternative approach to FMT. Additionally, Study II provides a **direct comparison of the efficacy and pre-FMT clinical characteristics between the enteric tube and oral capsule methods**. Research on upper GI FMT approaches is crucial to developing less invasive strategies for frail and polymorbid CDI patient populations without compromising treatment outcomes.

Despite the rising recognition of FMT as a treatment for CDI, major safety concerns remain unresolved. Currently, there is a lack of long-term safety data regarding FMT, particularly concerning the potential transmission of infectious, metabolic, oncological, or autoimmune diseases. This series of studies provides **clinical data on the long-term effects of gut microbiota modulation, including extended follow-up periods after FMT.** This research provides insights into outcomes over a long follow-up period and explores the long-term safety profile of both oral capsule and enteric tube FMT methods.

Aim and objectives

This study aims to evaluate the clinical effectiveness and long-term safety outcomes of fecal microbiota transplantation in treating recurrent *Clostridioides difficile* infection using different fecal microbiota transplantation delivery modalities.

Objectives:

1. To assess the efficacy of fecal microbiota transplantation using fresh donor feces administered via enteric tube in patients with recurrent *Clostridioides difficile* infection.
2. To assess the efficacy of fecal microbiota transplantation using frozen donor feces administered via oral frozen capsules in patients with recurrent *Clostridioides difficile* infection.
3. To compare the clinical cure rates between oral capsules and enteric tube fecal microbiota transplantation delivery methods.
4. To evaluate periprocedural and long-term safety of fecal microbiota transplantation delivered via the upper gastrointestinal route.

1. REVIEW OF THE LITERATURE

1.1. Epidemiology of *Clostridioides difficile* infection

Clostridioides difficile, formerly known as *Clostridium difficile*, is an anaerobic, gram-positive, spore-forming, toxin-producing bacillus [38]. It can be found in soil and the GI tracts of humans and animals. The bacterium is capable of forming spores that are resistant to drying, heating, and various chemical agents, including widely used disinfectants. It is well known that *C. difficile* spores are transmitted among humans through the fecal-oral route [39]. Spores are predominantly present in healthcare-associated environments but can also be found in the broader environment and food sources, enabling *C. difficile* to cause both nosocomial and community-acquired infections [40]. The presence of *C. difficile* in the human gut does not always result in active infection. Asymptomatic colonization is well-documented, occurring in 4–15% of healthy, disease-free adults, approximately 21% of hospitalized adults, and up to 15–30% of individuals in long-term care facilities [41, 42]. The issue of CDI gained significant attention after 2000 when data from hospitals in North America and Europe indicated rising disease rates. Epidemiological data revealed that in the United States alone, CDI directly causes nearly 14,000 deaths annually [10]. This notable change was driven by the emergence of the more virulent BI/NAP1/027 strain of *C. difficile*, characterized by higher toxin production, increased sporulation, and resistance to fluoroquinolones, and predominantly found in healthcare environments [43]. After acknowledging rising infection rates, measures were implemented to reduce hospital-related cases by introducing infection prevention protocols and antibiotic stewardship programs. By 2017, these efforts helped decrease the hospital-related CDI burden by 36%, while the community-acquired infection burden remained unchanged. CDI-related hospitalizations decreased by 24% by the end of 2017. However, issues remain, as first recurrence rates and mortality among hospitalized patients did not change during this period, signaling the need for new therapies, such as FMT, to reduce mortality and rCDI rates [44].

1.2. Pathogenesis of *Clostridioides difficile* infection

The microbiome of healthy individuals consists of approximately 4,000 different bacterial species, and these microorganisms serve as a protective barrier against invasive pathogens. *C. difficile* can colonize the large intestine but does not cause symptoms unless certain host factors are present – namely,

decreased colonization resistance and a weakened immune response [45]. Shortly after CDI rates began to rise, antibiotic use was identified as a major factor driving intestinal dysbiosis and the proliferation of *C. difficile* [46–48]. Evidence shows that a decreased population of the Bacteroides and Firmicutes phyla is a key factor contributing to the overgrowth of *C. difficile* [49]. Symptomatic patients may exhibit a wide range of disease severity, from mild diarrhea to fulminant colitis with megacolon and potentially fatal outcomes [50]. The clinical symptoms of CDI are directly caused by two primary toxins: *C. difficile* toxin A and toxin B [51]. Some more virulent strains, such as BI/NAP1/027, can also produce a third toxin, the binary toxin; however, testing for this toxin is not mandatory for CDI diagnosis [45, 52]. These toxins exert their effects on colonocytes by inducing apoptosis, disrupting the intestinal barrier, and triggering neutrophil-mediated colitis [18]. Toxins, rather than the bacteria themselves, trigger a systemic response to infection. They stimulate the production of tumor necrosis factor and interleukins, which are associated with inflammation and the formation of pseudomembranes in the colon [38].

1.3. Risk factors for *Clostridioides difficile* infection

Currently, three major and well-established risk factors for CDI are recognized: the most significant is recent antibiotic use, followed by advanced age (65 years or older) and exposure to healthcare environments [18, 40, 53–58]. Antibiotics and CDI disrupt the normal gut ecosystem. Studies show that patients with CDI have reduced populations of Bacteroidetes and Firmicutes, along with an increased population of Proteobacteria [59]. An additional mechanism of antibiotic-induced dysbiosis involves the interaction between the microbiota and bile acids [60]. Disrupted bile acid metabolism facilitates spore germination and enhances *C. difficile* toxin activity [61]. Furthermore, the concentration of primary bile acids may serve as a predictor of rCDI [62]. Although all antibiotics are considered risk factors, cephalosporins, fluoroquinolones, clindamycin, amoxicillin, and ampicillin are the primary contributors to dysbiosis that predisposes to CDI [18, 63–66]. Cephalosporins are a major driver of CDI outbreaks because these β-lactam antibiotics have been, and continue to be, the preferred antimicrobial agents for many common infections and are widely used in healthcare settings [63, 67]. The duration of antibiotic therapy is also an important modifiable factor that promotes more rational antibiotic use. A recent study showed that a 14-day antibiotic prescription increased the risk of CDI by 27% compared to a 7-day course [67]. Additionally, published data suggest that antibiotics with higher

activity against anaerobes (e.g., *Bacteroides*) are more potent in disrupting the gut microbiome and are associated with an even greater risk of CDI [68, 69]. In contrast, antibiotics such as tetracyclines have a relatively lower risk of inducing CDI. This property of tetracyclines may be advantageous for high-risk patients who require continuous antimicrobial therapy [67, 70].

Advanced age (> 65 years) is a non-modifiable risk factor for primary and rCDI and is associated with disease severity and worse outcomes [14, 51, 71, 72]. Microbiome studies suggest that intestinal bacterial diversity decreases in older individuals, making them more susceptible to colonization by pathogenic microorganisms [73, 74]. Another age-related factor is impaired immune response to infection. Geriatric patients exhibit decreased cellular and humoral immune responses, reducing their ability to combat infections [57, 75, 76]. According to data from the US registry, the prevalence of CDI significantly increases with age among hospitalized patients. In the age group of 65–79, CDI prevalence is 1.35%, increasing to 1.85% for people aged 80 and older [77]. A large study of 10,975 CDI cases revealed an overall 3-month mortality rate of 5.99%, with mortality jumping to 13.5% in the 80-year-old age group [78]. Contact with healthcare environments represents a significant risk factor for CDI. As previously noted, up to 30% of residents in long-term care facilities are colonized with *C. difficile*, and hospital admission in patients with pre-existing colonization increases the risk of developing CDI sixfold [41, 79]. Although the incidence of community-acquired CDI is increasing, healthcare-associated CDI continues to constitute a substantial global burden [44]. Apart from major risk factors, there are additional, albeit less significant, risk factors. Data from large studies indicate that various comorbidities increase the risk of CDI. The most commonly recognized ones include chronic kidney disease, heart disease, female sex, inflammatory bowel disease (IBD), obesity, and immunosuppression [14, 20, 22, 80–82].

1.4. *Clostridioides difficile* infection diagnosis and treatment

The diagnosis of primary and rCDI is based on clinical presentation, primarily characterized by diarrhea, in conjunction with stool testing. Currently, CDI testing is recommended only for patients presenting with new-onset diarrhea, defined as the passage of three or more unformed stools within a 24-hour period [14, 22]. Guidelines in Europe and the United States recommend a two-step approach for stool testing to diagnose *C. difficile* infection. The first step involves nucleic acid amplification testing (NAAT) or glutamate dehydrogenase (GDH) assays to detect *C. difficile* colonization. The

second step employs enzyme immunoassays (EIAs) to confirm the presence of toxins A and B, which are critical for diagnosing active infection [14, 83–85].

Additional clinical manifestations of CDI may include abdominal pain, fever, and malaise [68]. Approximately 10% of patients progress to fulminant colitis, which is characterized by a systemic inflammatory response, profuse diarrhea, ileus, organ failure, and, in some cases, sepsis [86]. Clinicians should remain vigilant for atypical presentations of fulminant CDI, which may involve peritonitis, severe abdominal pain, ileus with or without toxic megacolon, and organ failure, even in the absence of diarrhea [22, 72, 87]. Alternatively, a CDI diagnosis can be established based on the clinical presentation of the disease and endoscopic findings of pseudomembranes in the large intestine, confirmed by histopathological examination [83].

The initial episode of CDI is generally treated with oral antibiotics, while FMT is currently recommended for recurrent or refractory cases. Oral vancomycin and fidaxomicin remain the drugs of choice for most cases, except in fulminant disease, where vancomycin is still preferred [88–90]. The agreed duration of therapy for both drugs is 10 days, which is typically sufficient to resolve symptoms [22]. For fulminant CDI, also referred to as severe or complicated CDI, high-dose oral vancomycin combined with intravenous metronidazole is recommended [91]. The use of metronidazole monotherapy is limited to mild cases in low-risk patients and may be considered in resource-limited settings where vancomycin is unavailable [92]. The recommended duration of oral metronidazole therapy is up to 14 days, but it is not suitable for prolonged or repeated courses due to the risk of neurotoxicity [93, 94]. Once diarrhea and other clinical symptoms of infection have resolved, repeated testing to confirm cure is unnecessary. Studies have shown that more than 60% of patients may continue to test positive for *C. difficile* even after achieving complete clinical resolution [95, 96].

It is important to note that despite advances in conservative treatment options, approximately 1% of all CDI cases – and up to one-third of fulminant or severe cases – ultimately require surgical intervention [86, 97, 98]. Currently, there is no universally accepted algorithm to determine which patients would benefit more from surgical management versus continued medical therapy. However, published data suggest that early surgical intervention in fulminant and severe CDI cases may improve patient outcomes [99–101].

In clinical practice, surgical decisions are typically made on a case-by-case basis. Retrospective analyses have identified several factors associated with a higher likelihood of requiring surgery, including congestive heart failure, peripheral vascular disease, advanced age, and significant electrolyte imbalances [86, 102]. Another high-risk subgroup comprises patients with

IBD, particularly those receiving glucocorticoids or immunosuppressants, both of which are linked to poorer outcomes [103, 104]. A meta-analysis further demonstrated that patients with ulcerative colitis (UC) and concurrent CDI were twice as likely to require colectomy compared to UC patients without CDI [105]. In summary, bowel perforation remains the only absolute indication for surgical intervention in CDI. In all other cases, the decision to proceed with surgery should be individualized, weighing potential risks and benefits for each patient [72].

1.5. Recurrent and refractory *Clostridioides difficile* infection

Guidelines define rCDI as the recurrence of diarrhea with laboratory confirmation of toxigenic *C. difficile* strain within 8 weeks after completing initial treatment [14, 17, 20, 85, 106]. Published studies report that 10–30% of patients experience rCDI, with the risk increasing further with successive episodes [33, 34, 107–109]. Additionally, rCDI significantly increases mortality compared to patients without recurrence. Data from U.S. hospitals indicate that rCDI raises mortality by 33% within 180 days of diagnosis [110]. Recurrence can result from the same strain responsible for the initial infection or reinfection with a different strain [111, 112]. In clinical practice, distinguishing between these mechanisms is not feasible and does not impact treatment strategy. Based on current evidence, the preferred treatment for first-time rCDI includes a tapered or pulsed oral vancomycin regimen or a 10-day course of fidaxomicin. Although data slightly favor fidaxomicin, both regimens demonstrate sufficient efficacy and are suitable for managing recurrent disease [108, 109, 113, 114]. Conversely, metronidazole has shown inferior cure rates compared to vancomycin and should be avoided for rCDI treatment [107]. Additional drugs such as Bezlotoxumab (BEZ) have been developed to prevent infection relapses and reduce rates of rCDI. BEZ is a long-lasting monoclonal antibody that binds to toxin B, preventing damage to colonic cells and thereby reducing the risk of CDI recurrence [115–117]. Despite promising initial clinical trials, later studies identified that costly therapy is justified only for limited subgroup of rCDI patients. A *Post hoc* investigation concluded that only patients older than 65 years with major risk factors – such as second or later CDI episode, immunosuppression, and severe CDI – benefit from this medicine [118]. This approach has been adopted and remains recommended by CDI treatment guidelines, with additional caution for patients with a history of cardiovascular disease, as it has been associated with increased mortality through an unidentified mechanism [14, 22].

Patients who fail conventional antibiotic treatment for rCDI present a significant therapeutic challenge, as achieving sustained cure in this population remains difficult. Over the past decades, alternative treatment approaches, collectively referred to as fecal bacteriotherapy or FMT, have emerged. Historical records suggest the use of fecal material for the treatment of diarrhea as early as the 4th century; however, the first published evidence dates back to 1958, when Ben Eiseman successfully used fecal enemas to treat pseudomembranous colitis [119]. For several decades following this initial report, FMT did not garner significant interest, and only small case studies were published [120, 121]. However, at the beginning of the 21st century, the CDI epidemic gained momentum, eventually becoming the most prevalent nosocomial infection, characterized by high mortality and recurrence rates [18]. The rising incidence of rCDI renewed interest in FMT as an alternative treatment. By 2008, approximately 100 cases of FMT had been reported, with a promising success rate of nearly 90% [122]. In subsequent years, several gastroenterologists adopted FMT, primarily administering fecal material into the large intestine via colonoscopy. Early follow-up data from small case series suggested that FMT is an effective and safe procedure [123–126].

1.6. Fecal microbiota transplantation for recurrent and refractory *Clostridioides difficile* infection

In 2013, a Dutch research group led by van Nood published the first randomized controlled trial (RCT) comparing FMT with vancomycin monotherapy for the treatment of rCDI. This study demonstrated an 81% cure rate after a single FMT via a nasoenteric tube, compared to 31% in the oral vancomycin group [19]. Similar clinical cure rates were replicated in subsequent RCTs comparing FMT to placebo, vancomycin, and fidaxomicin. The efficacy of FMT after a single infusion ranged from 65% to 92%, with multiple FMTs increasing the cure rate to over 90% [19, 25–27]. The first large meta-analysis, published by Quaraishi et al. [32] included data from 1,973 patients across case studies and RCTs. The reported cure rate after a single FMT was 84%, increasing to 92% with multiple FMT procedures. This study also compared upper and lower gastrointestinal (GI) FMT delivery methods, with a slight preference for the lower GI route, where cure rates ranged from 92% to 97%, compared to 82% to 94% for the upper GI route [32]. As FMT usage increased, data from national registries provided further insight into real-world effectiveness. Large-scale data from North American and Danish databases reported cure rates of approximately 89% after a single FMT, further

confirming FMT as a viable treatment modality for rCDI [127, 128]. However, a systematic review and meta-analysis conducted by Tariq et al. reported a significantly lower cure rate for rCDI and refractory CDI in RCTs (67.7%) compared to open-label studies (82.7%) after a single FMT. Subgroup analysis further revealed that studies including only rCDI patients demonstrated a cure rate of 79%, whereas those that included refractory CDI patients reported a lower cure rate of 63.9% [129]. It is evident that further studies evaluating the efficacy of FMT are needed, particularly for the treatment of refractory infections.

The broader application of FMT has opened new frontiers in the treatment of fulminant CDI, which is refractory to standard antibiotic therapies. Early successful case reports utilizing single FMT, with cure rates ranging from 66% to 91%, encouraged the development of FMT protocols specifically targeting refractory CDI [130, 131]. Another major factor driving further investigation into FMT was the high mortality associated with surgical treatment for refractory CDI. This population is generally considered poor candidates for surgery, as postoperative mortality rates range from 38% to 80% [102].

Fischer et al. [132] were among the first to propose a sequential FMT protocol, in which oral vancomycin combined with repeated FMTs via colonoscopy achieved cure rates of 87%–100%. The survival rate was 95% at four weeks and 75% at 12 weeks. Similarly, Ianiro et al. [133] conducted an open-label randomized trial on refractory CDI, comparing a single FMT followed by 14 days of oral vancomycin with multiple FMTs followed by the same antibiotic regimen. The overall success rates were 75% for the single FMT group and 100% for the multiple FMT group.

Both studies utilized colonoscopy-guided FMT and aimed to repeat infusions until pseudomembranes in the colon were fully eradicated. An additional benefit of FMT in this population is the reduction in colectomy rates, as colectomy is associated with high mortality [134]. A single-center retrospective study analyzing data from 430 patients with refractory CDI before and after the implementation of an FMT program found that the introduction of FMT significantly reduced CDI-related mortality from 43.2% to 12.1% ($P < 0.001$) [135].

In summary, published data indicate that multiple FMTs combined with antibiotic therapy are essential for the successful treatment of refractory CDI. However, experts emphasize that patients with severe or fulminant CDI should be evaluated by a multidisciplinary team, including surgeons, particularly when colonic toxic megacolon, ischemic colitis, or perforation is suspected, to determine the optimal treatment strategy [14, 72].

1.7. Fecal microbiota transplantation safety

As the number of FMT procedures performed worldwide has increased, greater attention has been given to the safety of the procedure. Despite growing evidence supporting the high efficacy of FMT, data on its short- and long-term safety remain heterogeneous, and comprehensive systemic analyses are lacking [21]. However, published data indicate that the incidence of adverse events is low, with most reported events being minor and primarily limited to gastrointestinal symptoms, making FMT an acceptable therapeutic option for CDI treatment [35, 136, 137]. The most commonly reported adverse effects following FMT include nausea, abdominal discomfort, bloating, diarrhea, constipation, and fever [28, 32, 137–139]. In clinical practice, distinguishing between CDI-related symptoms and FMT-induced side effects can be challenging. However, most symptoms resolve spontaneously or with symptomatic treatment, and the benefits of FMT outweigh the potential discomfort [23].

It is important to address expert concerns regarding the use of FMT in immunosuppressed or immunocompromised patients experiencing rCDI. The clinical decision to use FMT in this patient population remains a subject of ongoing discussion, particularly due to the potential risks that may outweigh the benefits when compared to standard antibiotic therapy.

Current evidence suggests that fecal microbiota transplantation (FMT) can be cautiously considered safe in immunosuppressed individuals, with multiple studies reporting a low incidence of serious adverse events (SAEs) [140–142]. A multicenter study conducted in a cohort of solid organ transplant recipients demonstrated that FMT for refractory and recurrent CDI was well-tolerated, with an SAE rate of only 3.2% and no documented cases of bacteremia or fatal outcomes [143].

However, it is important to note that most observational studies exclude severely immunosuppressed patients, resulting in heterogeneous data and limited generalizability. The British Society of Gastroenterology and Healthcare Infection Society recommend exercising particular caution when considering FMT in this population. Nonetheless, they conclude that the currently available evidence supports the efficacy and presumed safety of FMT for the treatment of rCDI in selected immunocompromised patients [144].

The American Gastroenterological Association (AGA) has issued separate expert consensus statements regarding the use of FMT in patients with varying degrees of immunosuppression. FMT is considered safe and is recommended for the treatment of rCDI in patients with mild to moderate immunosuppression. However, the use of FMT in severely immunosuppressed individuals is strongly discouraged due to the lack of safety data in this

population [145]. According to the AGA, patients classified as severely immunocompromised include those receiving chimeric antigen receptor (CAR) T-cell therapy, hematopoietic cell transplantation, ongoing cytotoxic therapy for solid tumors or hematologic malignancies, those with advanced primary immunodeficiency, and individuals with untreated or advanced HIV infection. These groups are at significantly increased risk for life-threatening infections potentially associated with FMT products [146]. Given these concerns, it is recommended that treatment of rCDI in severely immunosuppressed patients focus on prolonged antibiotic therapy until immunosuppression is resolved, rather than FMT.

Another area of concern within the immunocompromised population is patients with coexisting IBD. Individuals with IBD are at an increased risk of developing CDI, and CDI in this population is associated with higher rates of mortality and colectomy compared to those with IBD alone [147, 148]. A large population-based study from Canada found that patients with IBD have a 4.8-fold higher risk of developing CDI, are typically younger at the time of diagnosis, more likely to acquire the infection in the community, and experience higher recurrence rates [149]. The study also noted that patients with ulcerative colitis and Crohn's disease had a comparable risk of infection.

Despite initial concerns about safety, current evidence suggests that FMT is safe for treating rCDI in patients with IBD [150–154]. Although some cases of IBD flare-ups following FMT have been reported, establishing a direct causal relationship is difficult, as CDI itself is a known trigger for exacerbating IBD symptoms [151, 154]. For this reason, testing for *C. difficile* is recommended in patients presenting with IBD flares, as timely and appropriate antibiotic therapy can improve the effectiveness of flare management. It is also important to emphasize that CDI diagnosis – and any subsequent consideration for FMT – should be based on a confirmed toxin-positive test. This is particularly relevant in IBD patients, who are frequently colonized by *C. difficile* without active infection [155].

A comprehensive meta-analysis conducted by Rapoport et al. [156] included 5,099 patients and analyzed the incidence of SAEs in FMT recipients. The study concluded that the overall rate of FMT-related SAEs is very low, at 0.65%. The most frequently reported SAEs included bacteremia (0.19%), aspiration pneumonia (0.27%), and bowel perforation (0.20%). Based on these findings, an expert panel determined that SAEs are rare and are primarily associated with inadequate screening of FMT products or procedural safety concerns during FMT administration [23]. Overall, FMT for rCDI appears to be a safe long-term therapeutic option. Only a few isolated cases of newly diagnosed conditions potentially linked to FMT have been reported [128, 137, 139, 154, 156–161].

Although safety concerns remain regarding the potential transmission of infections through FMT, documented cases of antibiotic-resistant bacteria (ARB) transmission are rare. A particularly concerning example involves two cases of post-FMT bacteremia in immunosuppressed patients caused by extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli, one of which resulted in fatal sepsis [146, 162]. Both cases were traced to a single donor who was initially deemed healthy and had been providing stool donations to a commercial stool bank.

Additional reports include the transmission of Shiga toxin-producing *E. coli* (STEC) and two cases of enteropathogenic *E. coli* (EPEC) following FMT [163, 164]. After this data was published the U.S. Food and Drug Administration (FDA) released safety alerts and advised additional donor feces screening [165]. In response to these findings, the FDA issued safety alerts and recommended enhanced donor stool screening protocols [166, 167]. Furthermore, data from two single-center studies suggest that even healthy, non-healthcare-associated donors may carry ARB [148, 149]. These findings highlight the critical importance of methodical donor screening, as currently outlined in FMT guidelines [21, 23, 168].

1.8. Fecal microbiota transplantation development and practical aspects

As FMT began gaining momentum due to rising positive outcomes in CDI treatment, several technical and logistical questions were raised by researchers, particularly regarding donor screening, stool preparation, donor selection, and the optimal route of delivery. Even now, with increasing numbers of FMT procedures being performed worldwide, there remains a lack of standardization and unified protocols across FMT centers.

Effective donor recruitment and thorough screening are critical components of a successful and safe FMT program. In Europe, similar to other human tissue donations, fecal donations are strictly regulated as a voluntary process [21]. From a practical standpoint, recruiting suitable donors is challenging due to stringent inclusion criteria and extensive screening requirements. Data from a prospective study reported that only 1.7% of enrolled candidates were ultimately identified as eligible fecal donors [169].

Additional challenges in the recruitment and donation process stem from societal stigma associated with feces, logistical difficulties related to sample collection and transportation, and potential behavioral impacts on donors [170]. Currently, there is insufficient evidence to clearly define what constitutes a “healthy” human microbiome, and this area remains a subject for

future research [171]. During the development of the best FMT practices, the concept of the "super donor" was proposed; however, there remains a lack of conclusive evidence to support the use of specific donors to enhance outcomes in CDI or IBD treatment [172, 173]. Consequently, there is no established definition of the "ideal" or "most effective" FMT donor, despite several studies attempting to correlate microbiome composition with FMT success [174, 175]. Furthermore, the previously common practice of recruitting recipient-related donors was shown to have no significant impact on FMT outcomes, thereby complicating the fecal donation process [176–178].

Another important aspect concerns the use of fresh versus frozen fecal material. Initially, freshly donated feces were used for every FMT procedure; however, later studies demonstrated that frozen feces are equally effective [150, 179–182]. Moreover, the use of frozen material offers additional safety advantages, such as allowing for extended stool testing, sample quarantine before administration, and significantly simplifying the logistics of fecal donation, thereby enabling the establishment of stool banks. In summary, based on the available evidence, experts currently recommend the use of universal donors from established stool banks, utilizing frozen and extensively tested fecal material as the most practical, safe, and effective approach [21, 144, 168].

1.9. Fecal microbiota transplantation and different routes of delivery

FMT can be administered using various delivery methods aimed at introducing fecal material into the GI tract, with the primary target being the large intestine. These delivery routes are generally categorized into upper and lower GI tract methods. Upper GI approaches include nasogastric or enteric tube infusion, fecal administration via gastroscopy, and ingestion of frozen or lyophilized oral capsules. Lower GI delivery methods consist of enema, sigmoidoscopy, and colonoscopy [35]. To date, most published studies have focused on colonoscopic administration, with fewer cases involving upper GI delivery methods [37]. FMT via colonoscopy offers several potential advantages. First, current comparative studies suggest that colonic delivery may be slightly more effective than alternative routes [179, 180, 183–186]. Additionally, in cases of refractory CDI or FMT failure, colonoscopy allows for direct visualization of the colonic mucosa and identification of persistent pseudomembranes, which may necessitate adjustments in the treatment regimen to achieve full resolution [187–189].

Despite these considerations, expert consensus supports the clinical viability of both upper and lower GI delivery methods, with the choice largely depending on the expertise and resources of the performing center [144]. One notable exception pertains to enema-based FMT, as emerging evidence suggests this method may be associated with lower efficacy in treating recurrent CDI. Therefore, enema administration should be considered only when other routes are unavailable or contraindicated due to patient condition [184, 190, 191].

Although colonoscopic FMT remains the most commonly used method in published studies, expert guidelines acknowledge that conventional FMT for rCDI can be effectively delivered via multiple routes [144, 145]. Emerging evidence cautiously suggests that upper GI modalities may offer comparable efficacy in the treatment of rCDI; however, further research is warranted to confirm these findings [37, 128, 180, 184, 186, 192].

Among upper GI approaches, oral capsule delivery represents a particularly promising option. The development of frozen or lyophilized oral FMT capsules enables treatment without the need for endoscopic procedures, potentially reducing both the risk of endoscopy-related complications and overall procedural costs [193]. Preliminary data from small-scale studies indicate that the efficacy of oral capsules may be non-inferior to other FMT delivery methods for treating rCDI [28, 185, 190, 194]. Earlier theoretical concerns regarding small bowel bacterial overgrowth have not been substantiated, and the safety profile of capsule-based FMT has generally been favorable [37, 142, 195]. Nonetheless, larger and more standardized studies are necessary to establish optimal manufacturing practices for oral capsules. Current literature remains heterogeneous, with variations in stool preparation, encapsulation techniques, and administration protocols, all of which may influence treatment outcomes.

1.10. Alternatives to fecal microbiota transplantation and future development

The recognition of CDI as a consequence of gut microbiota dysbiosis has led to increased interest in probiotic products, which are sometimes even recommended by gastroenterologists for primary prevention [196]. Despite generally favorable attitudes toward prebiotics, probiotics and probiotics current evidence does not support their use for either primary or secondary prevention of CDI. A key limitation is that probiotics are regulated as food supplements rather than pharmaceuticals, and manufacturers are often reluctant to invest in rigorous clinical trials [197]. A meta-analysis evaluating

probiotics for primary CDI prevention in elderly hospitalized patients concluded that there was no significant protective effect [198]. Evidence supporting the use of probiotics for the treatment of active CDI is even more limited. A Cochrane review analyzing four clinical trials found no reliable evidence to support the use of probiotics for CDI treatment [199]. Accordingly, current expert guidelines advise against the use of probiotics for both primary and secondary prevention of CDI due to the lack of robust supporting evidence [14, 17].

The expanding clinical application of FMT for *C. difficile* colitis has prompted pharmaceutical companies to develop standardized microbiota-based therapies. Currently, two live biotherapeutic products (LBPs) have been approved by the United States FDA for the prevention of recurrent CDI: Rebyota and Vowst [200, 201]. These approvals are based on clinical trials comparing the LBPs to standard antibiotic treatment. However, no studies to date have directly compared the efficacy of these LBPs to conventional FMT. Although live biotherapeutic products represent a promising new class of microbiota-targeted therapies, their clinical use remains limited to the North America market, and evidence supporting their effectiveness relative to traditional FMT remains insufficient [59].

The growing number of FMT performed worldwide and the successful adaptation of FMT for treating CDI-induced dysbiosis have stimulated interest in exploring its potential applications beyond CDI. Among these, UC has received the most research attention. Initial case series reported encouraging outcomes, which helped drive the development of FMT programs targeting IBD [202, 203]. Subsequently, randomized controlled trials (RCTs) published promising preliminary results, suggesting therapeutic potential in UC [204, 205].

In the context of UC, two main therapeutic goals have emerged: induction of remission and maintenance of remission. Most studies to date have focused on inducing remission, with clinical trials comparing FMT to placebo, standard-of-care therapy, autologous FMT, or modified diets [205–208]. These trials generally suggest that patients receiving FMT are more likely to achieve clinical remission compared to controls. However, findings regarding the induction of endoscopic remission and the incidence of serious adverse events remain inconclusive. A smaller subset of RCTs has explored the role of FMT in maintaining remission in UC [207, 209]. In these studies, patients received FMT in combination with standard therapies, including mesalamine, methotrexate, thiopurines, or biologic agents. The outcomes related to sustained remission, safety, and quality of life were variable, and the evidence remains insufficient to support definitive conclusions.

While current evidence on the efficacy of FMT in UC is promising, it remains unclear which patient subgroups are most likely to benefit from this therapy or how FMT should be integrated into existing treatment algorithms. Published studies have demonstrated considerable methodological variability, employing different administration routes (upper *vs.* lower gastrointestinal tract), dosing regimens, treatment durations, and outcome measures. One of the key challenges is the limited understanding of microbiome engraftment dynamics in UC. Unlike patients with *C. difficile* colitis, who typically present with antibiotic-induced dysbiosis, UC patients do not exhibit uniform microbial disruption. This fundamental difference complicates the interpretation of engraftment success and its therapeutic implications [174]. Additionally, engraftment is likely influenced by multiple donor- and recipient-related factors, including genetics, baseline microbiome composition, comorbidities, diet, and concomitant medications [210]. Given the current gaps in knowledge, there is no formal recommendation to use FMT for either induction or maintenance of remission in UC. International guidelines advise restricting FMT use in UC to clinical trial settings rather than routine clinical practice [144, 145]. A recently published expert consensus on the application of FMT in IBD similarly concluded that the evidence remains insufficient and emphasized the need for more robust, standardized clinical trials to better assess FMT efficacy in this population [211].

Earlier research on the gut microbiome in Crohn's disease (CD) sparked interest in the potential of microbiota modulation as a therapeutic strategy for this chronic autoimmune condition. While the exact etiology of CD remains unclear, current evidence suggests that environmental and genetic factors, along with an impaired immune response to the gut microbiota, may contribute to disease pathogenesis [212]. Microbiome studies in CD patients have shown a relative increase in pro-inflammatory bacterial species and a reduction in anti-inflammatory microbes when compared to healthy individuals [213, 214]. Despite these findings, clinical evidence supporting the role of FMT in inducing or maintaining remission in CD remains limited. To date, only one randomized controlled trial has evaluated FMT for the maintenance of remission in CD. This trial found no significant benefit of FMT compared to placebo in sustaining clinical remission [215]. In summary, current evidence does not support the routine use of FMT for the treatment of CD. Expert guidelines recommend that FMT in CD should be limited to the context of clinical trials and not applied in standard clinical practice [144, 145].

Irritable bowel syndrome (IBS) is one of the most common conditions encountered in gastroenterological clinical practice. It is a complex disorder involving multiple pathophysiological mechanisms, including alterations in

the gut microbiome and dysfunction of the gut–brain axis [216]. IBS is classified as a functional gastrointestinal disorder and is defined by the presence of abdominal pain or discomfort associated with altered bowel habits, in the absence of any identifiable organic pathology [217]. Due to the heterogeneous and multifactorial nature of IBS, available treatment options often show limited long-term efficacy. This therapeutic gap has drawn attention to FMT as a potential intervention. To date, FMT for IBS has been investigated in several RCTs [218–220]. However, these studies exhibit a high degree of methodological variability, including differences in donor selection (single *vs.* multiple donors), route of administration (e.g., colonoscopy, oral capsules, or duodenal infusion), and outcome assessment. Some trials have reported improvements in quality of life among IBS patients following FMT, but these findings have not been consistently replicated, and the overall efficacy of FMT in this context remains uncertain [221, 222]. As of now, FMT for the treatment of IBS is not recommended for routine clinical use and should be restricted to clinical trials, as current guidelines emphasize the lack of sufficient evidence to support its widespread application [223, 224].

2. MATERIALS AND METHODS

2.1. Ethics statement

Both studies presented in this dissertation were approved by the Kaunas Regional Biomedical Research Ethics Committee (2011-03-08 Protocol No: BE-2-10, 2018-06-05 Protocol No: P2-BE-2-31/2018). Written informed consent was obtained from all participants.

2.2. Study I design and methodology

Study I was a consecutive case series that included patients with rCDI or refractory *C. difficile* infection who received FMT using fresh donor feces delivered via an enteric tube. All patients were treated at the Hospital of Lithuanian University of Health Sciences Kauno klinikos. FMT procedures were performed in the Department of Gastroenterology, and some patients were referred from regional hospitals specifically for FMT.

Inclusion criteria. Eligible participants were adults diagnosed with rCDI, defined as experiencing a second or subsequent episode of CDI, or with refractory CDI, defined as failure to respond to *C. difficile*-targeted antibiotic therapy. Diagnosis was based on clinical presentation and confirmed by stool testing.

The key clinical criterion for CDI diagnosis was diarrhea (≥ 3 unformed stools within 24 hours). Additional symptoms such as abdominal pain, nausea, fever, and elevated inflammatory markers (leukocytosis and C-reactive protein) were considered supportive but not mandatory. Laboratory confirmation of CDI was performed using enzyme-linked immunosorbent assay (ELISA) to detect enterotoxins A and B in stool samples (Simple 2a-bdiff / Stick 2a-bdiff, Operon, Spain), ensuring detection of toxigenic *C. difficile* strains.

Patients were excluded from the study if they were unable to provide informed consent and had no legal guardian, had confirmed infections with other enteric pathogens known to cause diarrhea, or presented with neutropenia (defined as < 500 cells/mm^3). Additional exclusion criteria included documented food allergy associated with anaphylaxis, ongoing systemic antibiotic therapy for other indications that could not be discontinued, presence of ileus or toxic megacolon, or fulminant CDI – characterized by a white blood cell count > 30,000/mL, temperature exceeding 40 °C, septic shock, hypotension, or positive peritoneal signs. Patients were also excluded

if they were under 18 years of age, pregnant or breastfeeding, or unwilling to comply with the study protocol.

The primary outcome was the efficacy of FMT, defined as the absence of recurrent diarrhea within eight weeks following the procedure. This endpoint was used to evaluate the success of FMT in resolving CDI.

Secondary outcomes included an assessment of FMT safety, which involved monitoring for periprocedural complications such as gastrointestinal symptoms, allergic reactions, or infections. Long-term safety was evaluated through follow-up for any new medical diagnoses or deaths potentially attributable to FMT. Additionally, a comparative analysis between responders and non-responders was conducted to explore clinical and demographic factors that may predict reduced FMT efficacy.

Sample size calculation. This study aimed to evaluate both the primary efficacy of FMT for treating rCDI and compare outcomes between responders and non-responders. Baseline recurrence rate (P_0): Estimated at 40%, based on previous outcomes with standard antibiotic therapy. Expected success rate with FMT (P_1): 80%, based on published data (range: 65%–92%). Significance level (α): 0.05. Power ($1 - \beta$): 80%. Sample size was estimated using a one-sample proportion test, requiring 23–30 patients to detect a statistically significant effect. For subgroup analysis between responders and non-responders, an estimated cohort of 30 patients (24 responders and 6 non-responders) would be sufficient for exploratory comparison. However, a total of 40–50 patients was recommended to improve statistical reliability.

Study population and follow-up. In total, 60 consecutive patients were enrolled in Study I. Patient inclusion began in December 2015 and concluded in October 2019. All FMT procedures were performed in the Department of Gastroenterology at Kauno klinikos. Initial CDI treatment was administered in local or regional hospitals prior to referral.

All patients were followed until September 1, 2020. An overview of the study design is presented in Fig. 2.2.1.

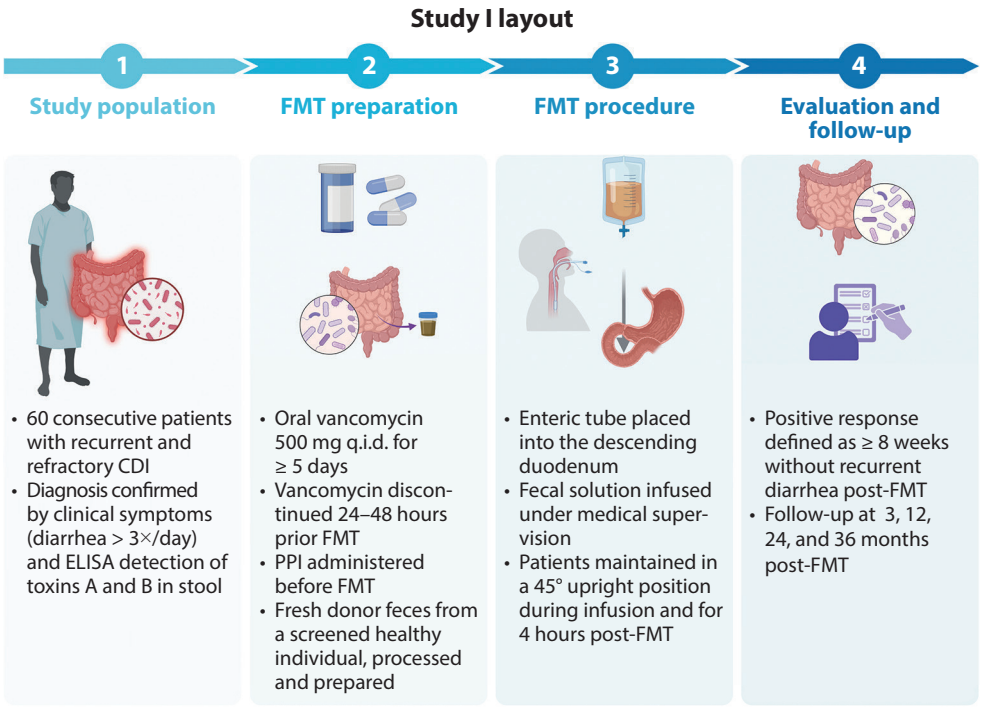


Fig. 2.2.1. *Schematic representation of the Study I*

2.3. Study II design and population

Study II was a comparative cohort study that included 60 patients diagnosed with rCDI who underwent FMT using frozen donor feces. Thirty patients received FMT via oral frozen capsules, and their outcomes were compared to those of 30 patients who received FMT via enteric tube.

During the inclusion process, patients were offered the option to receive FMT via oral capsules. No randomization was performed. Patients who declined or were unable to undergo oral capsule administration received FMT via enteric tube as per the standard protocol at the Hospital of Lithuanian University of Health Sciences Kauno klinikos.

Inclusion criteria. Eligible participants were patients with confirmed rCDI, defined as the occurrence of a second or subsequent CDI episode. Diagnosis was based on clinical presentation and laboratory confirmation. The primary clinical criterion was diarrhea (≥ 3 unformed stools within 24 hours). Additional symptoms such as abdominal pain, nausea, fever, and elevated inflammatory markers (leukocytosis and C-reactive protein) were considered supportive indicators. Laboratory confirmation of CDI was performed using an ELISA assay to detect enterotoxins A and B in stool samples

(Simple 2a-bdiff / Stick 2a-bdiff, Operon, Spain), confirming the presence of a toxigenic *C. difficile* strain.

Patients were excluded from the study if they were unable to provide informed consent and had no legal guardian, had confirmed infections with other enteric pathogens known to cause diarrhea, or presented with neutropenia (defined as < 500 cells/mm³). Additional exclusion criteria included documented food allergy associated with anaphylaxis, ongoing systemic antibiotic therapy for other indications that could not be discontinued, presence of ileus or toxic megacolon, or fulminant CDI – characterized by a white blood cell count > 30,000/mL, temperature exceeding 40 °C, septic shock, hypotension, or positive peritoneal signs. Patients were also excluded if they were under 18 years of age, pregnant or breastfeeding, or unwilling to comply with the study protocol.

The primary objective of the study was to compare the efficacy of FMT administered via oral capsules versus enteric tube. Treatment success was defined as the absence of recurrent diarrhea within eight weeks following FMT. A direct comparison of clinical response rates, as well as both short- and long-term safety profiles, was conducted between the two groups.

Secondary outcomes included a comparison of baseline patient characteristics – specifically age, gender, immunosuppression status, and comorbidities – between the oral capsule and enteric tube cohorts. Safety assessment focused on identifying any periprocedural adverse events and evaluating long-term outcomes, including new medical diagnoses potentially related to FMT and any FMT-associated mortality.

Sample size calculation. Sample size determination was based on a comparison of FMT efficacy between the two upper GI administration methods and an analysis of baseline demographic characteristics. Assuming a baseline CDI recurrence rate of 40% and an expected FMT success rate of approximately 80%, a two-sample proportion test was used with a significance level of 0.05 and statistical power of 80%. Based on these parameters, a minimum of 40–50 patients per group was considered appropriate to ensure sufficient statistical power for comparing treatment outcomes and demographic factors.

Study population and follow-up. Study II included a total of 60 patients treated at the Hospital of Lithuanian University of Health Sciences Kauno klinikos, between 2017 and 2021. All FMT procedures were conducted in the Department of Gastroenterology. Initial CDI treatment was administered in local or regional hospitals prior to patient referral. Of the 60 patients, 30 received FMT via oral capsules, and 30 underwent FMT using the standard enteric tube method. All patients were followed for a period of six months after FMT. The study layout is illustrated in Fig. 2.3.1.

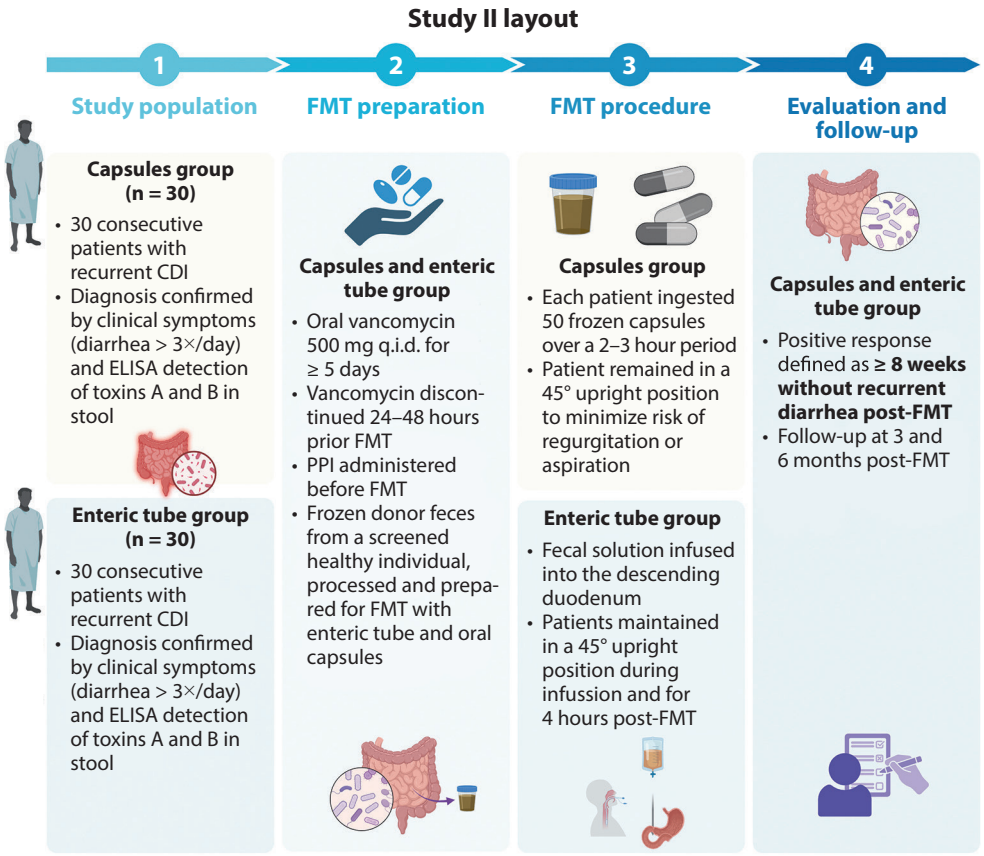


Fig. 2.3.1. *Schematic representation of Study II*

2.4. Recipients preparation before fecal microbiota transplantation

CDI patient preparation was standardized across both studies and was identical for both the enteric tube and oral FMT methods. Prior to the initial FMT, all recipients received standard-of-care CDI treatment with oral antibiotics. The majority of patients were treated with oral vancomycin for 10 days as per standard protocol. However, some patients from regional hospitals received metronidazole due to the unavailability of vancomycin at their treatment site.

To ensure a more uniform CDI treatment before FMT and to facilitate proper bacterial engraftment, all recipients were administered oral vancomycin 500 mg four times daily (q.i.d.) for a minimum of five days prior to FMT. In accordance with our center's FMT protocol, to mitigate the potential impact of gastric acid on the infused or ingested material, all recipients

received 40 mg of omeprazole the evening before and the morning of the FMT procedure. Oral vancomycin was discontinued at least 24 hours – but no more than 48 hours – before the intervention to allow for adequate antibiotic washout from the large intestine. No patients underwent bowel preparation, as FMT was administered via the upper GI route in all cases.

2.5. Donor preparation and screening

Donor selection, evaluation, as well as blood and fecal examination were conducted in accordance with published guidelines and were updated as new recommendations emerged [19, 21, 35, 165, 225]. The donor screening program followed a standardized protocol at our center, as previously described in published studies [226]. A total of four unrelated donors participated in fecal donation. In all cases, only a single donor's feces were used for each FMT procedure. Donations were entirely voluntary, with no financial compensation provided.

All donors were male, younger than 35 years, with a Body Mass Index (BMI) between 18.5 and 24.9 kg/m², and without preexisting diseases or contraindications for stool donation, as outlined in the stool banking consensus [21]. Briefly, on the day of donation, donors were required to have no history of antibiotic, immunosuppressant, or chemotherapy use in the past six months, no history of infectious disease in the past three months, and no chronic use of proton pump inhibitors (PPIs) for more than three months. All donors underwent comprehensive serological and microbiological blood and fecal screening. Blood tests included screening for hepatitis A, B, and C viruses, human immunodeficiency virus (HIV-1 and HIV-2), *Treponema pallidum*, *Strongyloides stercoralis* (nematodes), complete blood cell count, bilirubin, creatinine, and aminotransferases. Additionally, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) screening was performed, as FMT was also administered to immunocompromised patients.

Stool testing included screening for common pathogens such as *C. difficile*, *Shigella*, *Salmonella*, *Yersinia*, *Campylobacter*, norovirus, rotavirus, adenovirus, *Giardia lamblia*, *Cryptosporidium parvum*, protozoa, helminths, and parasites. As new concerns regarding ARB emerged, additional screening was implemented [165]. Donor feces were tested for multidrug-resistant organisms, including extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, carbapenem-resistant *Enterobacteriaceae* (CRE), vancomycin-resistant enterococci (VRE), and methicillin-resistant *Staphylococcus aureus* (MRSA). A summary of the screening tests is presented in Fig. 2.5.1.

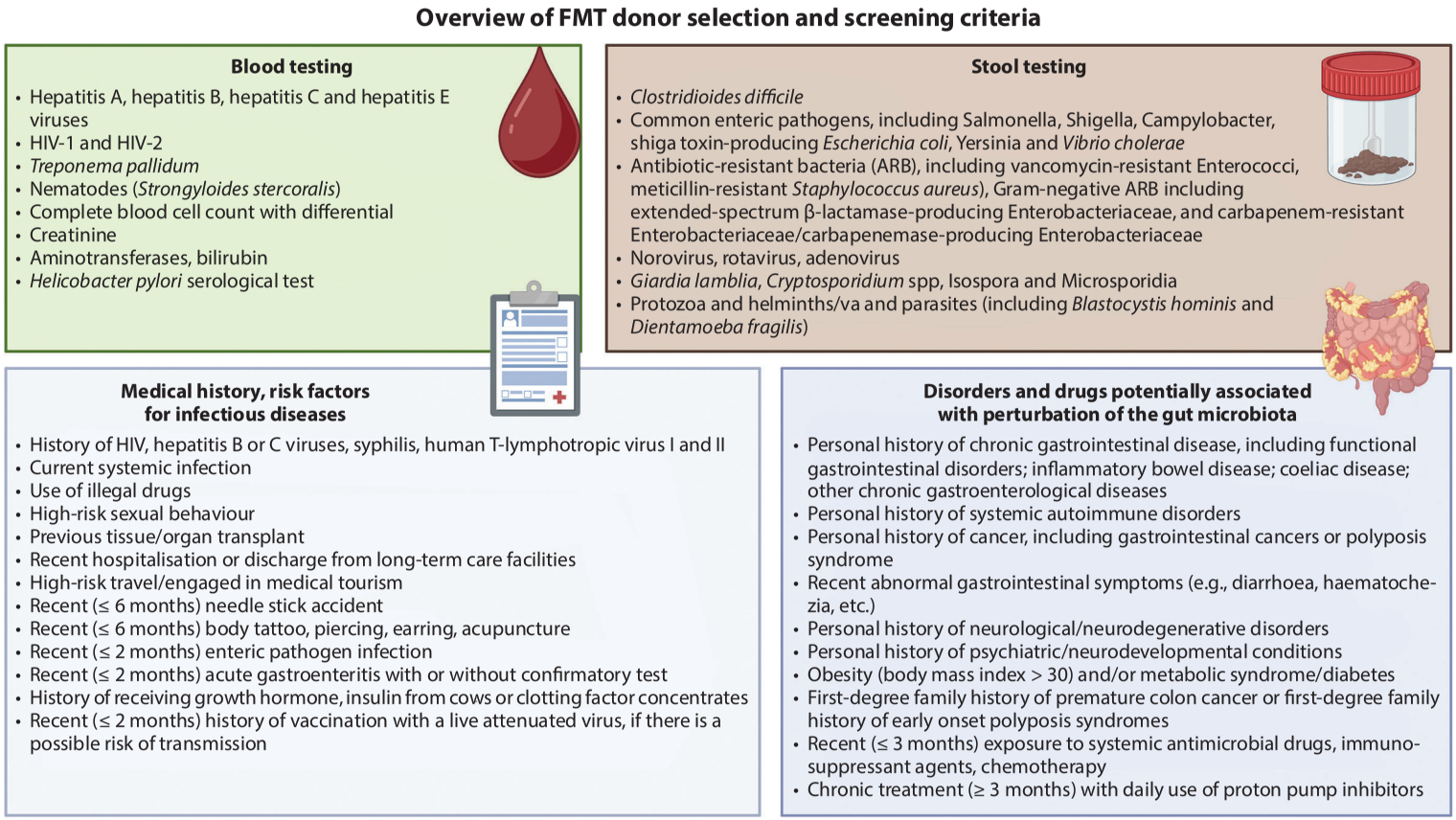


Fig. 2.5.1. *Donor screening protocol*

Adapted from International consensus conference on stool banking for feacal microbiota transplantation in clinical practice [21].

Across both studies, fecal material from different donors was used, and individual donors contributed at multiple time points. Donor stool testing was repeated every two months during active donation periods to ensure ongoing safety and compliance with screening protocols.

2.6. Stool preparation

In Study I, fresh feces were used. Stool samples were collected in designated disposable containers and stored at 4 °C. The time between stool collection and preparation did not exceed six hours. Further processing was conducted under a fume hood to ensure operator safety and sample sterility.

For each FMT procedure, 50 g of donor feces was blended with 150 mL of 0.9% NaCl (saline solution). The resulting slurry was then filtered to remove solid particles, and an additional 0.9% NaCl was added to achieve a final volume of 500 mL. The prepared solution was transferred into a specialized bag, covered with dimmed packaging to prevent exposure to direct sunlight, which could potentially affect the viability of fecal material.

Study II was conducted exclusively using frozen fecal material. Emerging data suggested that frozen material offers improved safety due to the ability to quarantine and test the sample before FMT [21]. Additionally, studies have demonstrated that frozen fecal transplants are equally effective as fresh material FMT [24). For this study, 50 g of donor feces was used for each FMT procedure in both the nasoenteric tube and capsule groups. Stool samples were collected in disposable containers and stored at 4 °C for no longer than six hours before processing. All handling was performed under a fume hood. The fecal material was blended with 0.9% NaCl (saline solution) to ensure homogenization, then filtered to remove solid particles. The prepared fecal solution was mixed with glycerol and deep-frozen at –80 °C for storage. For the enteric tube method, the frozen material was further diluted with 0.9% NaCl to reach a final volume of 500 mL and transferred into a specialized dimmed bag, as described previously.

The manufacturing of oral capsules was also performed under a fume hood. The deep-frozen fecal solution was thawed and centrifuged twice to separate the supernatant from the sediment. The sediment was then encapsulated using DRcaps® capsules (*Lonza Group, Switzerland*). Oral FMT was delivered using double DRcaps® acid-resistant, enteric-release capsules to protect the fecal material from degradation in the gastric environment. Each 50 g fecal sample yielded 50 oral capsules. The final product was stored at -20 °C until administration.

2.7. Fecal microbiota transplantation procedure

The enteric tube FMT procedure is a standard method in our center, and this approach was consistently applied in both studies. First, an upper GI endoscopy was performed, during which an enteric tube (*Kangaroo™ Nasogastric Feeding Tube, Cardinal Health, USA*) was placed directly into the descending duodenum under direct visualization by the endoscopist. To confirm the proper tube placement, an abdominal X-ray was performed before each FMT.

The prepared fecal solution bag was then connected to the enteric tube. During the infusion, patients remained in a 45° upright position in bed. To minimize the risk of aspiration, recipients were required to maintain this position for at least four hours post-procedure. Throughout the FMT infusion, all patients were closely monitored by medical staff, and post-procedure observation continued for six hours. After completing the FMT, the nasoenteric tube was flushed with 20 mL of water and removed to enhance comfort of patients.

In Study II, 30 patients underwent FMT via oral frozen capsules. Each patient ingested 50 units of frozen capsules within a single day, with administration taking place over a 2–3 hours period. To minimize the risk of regurgitation or aspiration, patients were positioned in a 45° upright position throughout the ingestion process. Medical staff supervised the capsule intake and continued monitoring for six hours post-FMT.

2.8. Evaluation of outcomes

2.8.1. Response to fecal microbiota transplantation and primary efficacy

In Studies I and II, FMT efficacy was determined by the absence of clinical CDI symptoms. According to the guidelines, the resolution of diarrhea following FMT was the primary indicator of a positive therapeutic response [17, 23, 85, 106]. Primary non-responders were defined as patients who failed to show clinical improvement within one week and continued to experience diarrhea. Patients who remained free of recurrent diarrhea for at least eight weeks were classified as cured of CDI. Clinical FMT responders were not routinely tested for *C. difficile*, as post-FMT testing is considered clinically irrelevant and is not recommended by any established guidelines [35].

2.8.2. Follow-up after fecal microbiota transplantation

In Study I, patients were followed from the day of FMT until September 1st, 2020. To assess the long-term safety profile of FMT, follow-up data were collected on rCDI episodes, early and late complications, and overall health status at 3, 12, 24, and 36 months post-FMT, where available, as well as at the end of the follow-up period. In Study II, all patients were monitored for a fixed period of six months. The majority of follow-ups were conducted via telephone interviews, while a smaller number of patients had outpatient consultations with a gastroenterologist. During follow-up, data on adverse events, changes in health status, and the onset of new gastrointestinal symptoms, particularly diarrhea, were collected. In the later follow-up period, patients were also surveyed regarding new diagnoses of infectious, autoimmune, oncological, or metabolic diseases.

2.8.3. Statistical analysis

Categorical variables, including gender distribution, multimorbidity, and immunosuppressant use, were compared using the Chi-square test. Continuous variables, such as patient age and the number of previous CDI episodes, were compared between groups using the independent samples t-test. For variables with very small sample sizes, such as the presence of IBD, ulcerative colitis, Crohn's disease, immunosuppressed status, glucocorticoid use, and biological therapy use, Fisher's exact test was applied. All statistical analyses were performed using *IBM SPSS Statistics for Windows*, *Version 30.0* (*IBM Corp., Armonk, NY, USA*).

3. RESULTS

3.1. Clinical data of the Study I cohort and fecal microbiota transplantation recipients

Study I included 60 consecutive patients who underwent FMT for recurrent or refractory CDI. Patient demographics and clinical characteristics are summarized in Table 3.1.1. The median patient age was 72.5 years (range: 32–99 years). The gender distribution was nearly equal, with 28 females (46.7%) and 32 males (53.3%). The mean number of prior CDI episodes before FMT was 2.7 ± 1.3, ranging from one to seven episodes. Among the study population, nine patients (15%) were receiving immunosuppressive therapy for comorbid conditions, including glucocorticoids, azathioprine, methotrexate, tacrolimus, or mycophenolate mofetil, and continued these treatments after FMT. The majority of patients (83.3%, n = 50) had multiple comorbidities, defined as the presence of two or more chronic diseases. FMT was selected as the therapeutic approach for patients experiencing a second or later recurrence of CDI or those who had failed to respond to standard antimicrobial treatments.

Table 3.1.1. *Demographic and clinical characteristics of FMT recipients in Study I*

Variable		**FMT patients (n = 60)**
Age (years)	Median	72,5
	Range	[32–99]
Gender distribution	Female	28 (46.7%)
	Male	32 (53.3%)
Previous episodes of CDI	Mean ± SD	2.7 ± 1.3
	Range	[1–7]
Use of immunosuppressants		9 (15%)
Multimorbidity		50 (83.3%)

3.2. Treatment efficacy of fecal microbiota transplantation in Study I

3.2.1. Results after first fecal microbiota transplantation

The findings indicate that 48 out of 60 patients achieved full remission following a single FMT, resulting in an overall cure rate of 80%. These 48 responders remained disease-free for at least eight weeks post-FMT. Among

the 60 recipients, 12 patients experienced recurrent symptoms or failed to show clinical improvement after FMT. In this study, early non-responders were defined as patients who continued to have diarrhea within the first week following FMT. Treatment failure was classified as the recurrence of CDI symptoms within eight weeks of the initial FMT.

3.2.2. Results after second fecal microbiota transplantation

As 12 patients experienced recurrent diarrhea after the first FMT, the recommended clinical approach was applied, and a repeat FMT was performed. Prior to the second transplantation, all non-responders received standard CDI treatment with oral vancomycin, ensuring a minimum of five days of vancomycin 500 mg q.i.d. before the procedure. The transplantation method remained unchanged, with all 12 recipients undergoing FMT via an enteric tube. Ten out of 12 patients achieved full resolution of diarrhea following the second FMT, resulting in an overall cure rate of 96.7% in this cohort.

3.2.3. Results after third fecal microbiota transplantation

The remaining two patients experienced recurrent diarrhea following the second FMT. One non-responder had multiple comorbidities, including type 2 diabetes, stage 4 chronic kidney disease, Parkinson's disease, hypothyroidism, chronic obstructive pulmonary disease, and ischemic heart disease. After the failure of the second FMT, alternative causes of diarrhea were ruled out, and a third FMT was scheduled following at least a five-day course of oral vancomycin 500 mg q.i.d. In the second patient, CDI recurrence was likely associated with the use of broad-spectrum antibiotics for pneumonia and skin infection, with rCDI diagnosed shortly after antibiotic treatment. Both patients underwent a third FMT after completing a course of vancomycin 500 mg q.i.d. and achieved sustained cure within eight weeks, resulting in a final overall cure rate of 100%. Cure rates are shown in Fig. 3.2.3.1.

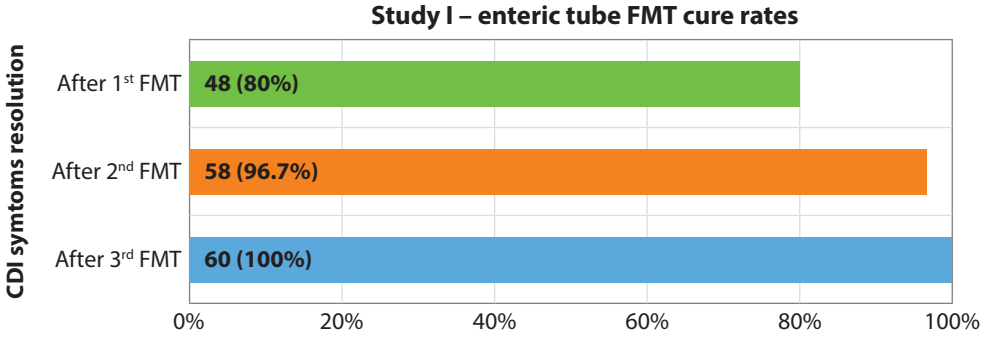


Fig. 3.2.3.1. *Cure rates following enteric tube FMT in Study I*

3.2.4. Comparison of fecal microbiota transplantation responders and non-responders

Patients who responded to a single FMT were compared to those who experienced rCDI following transplantation, as presented in Table 3.2.4.1. No significant differences were found in age ($P = 0.124$), gender distribution ($P = 0.697$), the number of previous CDI episodes ($P = 0.804$), immunosuppressant use ($P = 0.365$), or the proportion of polymorbid patients ($P = 1$).

Table 3.2.4.1. *FMT responders and non-responders comparison*

Variable		**FMT responders (n = 48)**	**FMT non-responders (n = 12)**	***P* value**
Age (years)	Mean ± SD	71.8 ± 12.9	61.7 ± 19.5	0.124
	Range	[37–99]	[32–85]	
Gender distribution	Female	27 (56.3%)	6 (50%)	0.697
	Male	21 (43.6%)	6 (50%)	0.697
Previous episodes of CDI	Mean ± SD	2.6 ± 1.3	2.6 ± 1.3	0.804
	Range	[1–7]	[1–5]	
Use of immunosuppressants		6 (12.5%)	3 (25%)	0.365
Multimorbidity		40 (83.3%)	10 (83.3%)	1

3.3. Follow-up data from Study I

3.3.1. Early adverse events following fecal microbiota transplantation

Mild, non-life-threatening adverse events are commonly expected shortly after the FMT procedure. A few patients reported transient symptoms such as mild nausea, abdominal discomfort, and increased bowel movements, all of which were resolved within a few hours without requiring medical intervention. These minor side effects were not systematically documented, as distinguishing between CDI-related symptoms and FMT-related effects is challenging. Moreover, these symptoms had no significant impact on the recipient's health and were resolved with minimal intervention. One patient experienced a low-grade fever 12 hours after FMT but did not require antipyretic medication. Despite this transient fever episode, the patient achieved a sustained cure following a single FMT, indicating that this event did not impact treatment efficacy. No adverse events directly related to endoscopic procedure or FMT administration were observed in this study.

3.3.2. Long-term follow-up data and adverse events

The median follow-up time after FMT in Study I was 20 months (range: 1–55 months). Detailed follow-up data and recorded adverse events are presented in Table 3.3.2.1.

Table 3.3.2.1. *Long-term follow-up outcomes after FMT in Study I*

Variable		n	%
Periprocedural adverse events	Regurgitation of donor feces or vomiting	0	0
	Fever after FMT	1	1.67
	Pneumonia after FMT	0	0
	Endoscopy related events	0	0
Adverse events at 12 weeks of follow-up	Other probably FMT related events	1	1.67
	Deaths (FMT related)	0	0
	Deaths (not FMT related)	3	5
Adverse events at the end of the follow-up period	Median follow-up time in months (range)	20	1-55
	New onset oncologic diseases	0	0
	New onset cardiovascular diseases	0	0
	New infectious diseases	0	0
	New onset metabolic diseases	0	0
	Deaths (not FMT related)	6	10

It is important to note that three patients died within the first eight weeks following FMT. However, all had severe comorbidities, and their deaths were primarily attributed to preexisting conditions unrelated to CDI or the FMT procedure. One case of dynamic ileus was reported three weeks after FMT, though it was unlikely to be associated with the treatment. This condition was managed conservatively, and the patient fully recovered. Notably, this patient achieved sustained cure after a single FMT, with no rCDI diagnosed during the follow-up period. In summary, no SAEs or FMT-related deaths were observed in Study I. A total of six patients died during the follow-up period, with all fatal outcomes linked to underlying severe comorbidities.

3.4. Study II cohort and patient data

Study II included 30 patients who underwent FMT via oral capsules, and their clinical data were compared to 30 patients who received FMT through an enteric tube for the treatment of rCDI. The mean age in the capsule group was 66.03 ± 20.69 years, compared to 66.23 ± 17.89 years in the enteric tube group. In both groups, there was a slight predominance of female participants,

with 16 females (53%) in the capsule group and 18 females (60%) in the enteric tube group. More than half of the patients in both groups were classified as polymorbid, defined as having two or more chronic diseases. Specifically, 17 patients (56.7%) in the capsule group and 19 patients (63%) in the enteric tube group were identified as polymorbid.

Additionally, both groups included six patients with a pre-existing diagnosis of IBD prior to the diagnosis of rCDI. The number of immunosuppressed patients was equal between groups, with eight immunosuppressed individuals in each. However, the types of immunosuppressive agents used varied between patients, as detailed in Table 3.4.1. After statistical analysis no significant differences were observed between the groups in terms of age ($P = 0.97$), gender distribution (males: $P = 0.82$, females: $P = 0.66$), multimorbidity ($P = 0.79$), the presence of IBD ($P = 1$), or immunosuppression status ($P = 1$).

Table 3.4.1. *Clinical and demographic characteristics of FMT recipients in Study II*

Variable	FMT capsules (n = 30)	FMT nasoenteric tube (n = 30)	*P* value
Age, mean ± SD	66.03 ± 20.69	66.23 ± 17.89	0.97
Males	14 (47%)	12 (40%)	0.82
Females	16 (53%)	18 (60%)	0.66
Multimorbidity	17 (56.7%)	19 (63%)	0.79
IBD	6 (20%)	6 (20%)	1
Ulcerative colitis	4 (13.33%)	5 (16.67%)	1
Crohn's disease	2 (6.67%)	1 (3.33%)	1
Immunosuppressed	8* (26.67%)	8** (26.67%)	1
Glucocorticoids	5 (16.67%)	8 (26.67%)	0.55
Other immunosuppressants	2 (6.67%)	5 (16.67%)	0.43
Biological therapy	4 (13.33%)	0 (0%)	0.12

* 3 patients received more than 1 immunosuppressant drug; ** 5 patients received more than 1 immunosuppressant drug.

3.5. Fecal microbiota transplantation treatment effectiveness in Study II

3.5.1. Efficacy after first and second fecal microbiota transplantation

In the oral capsule group, 22 out of 30 patients achieved clinical cure after a single FMT, resulting in a primary cure rate of 73.3%. Similarly, in the enteric tube group, 24 out of 30 patients remained free of recurrent symptoms, yielding an 80% primary cure rate. The difference in treatment efficacy between the two groups was not statistically significant ($P = 0.97$). Recurrent infection within eight weeks post-FMT was diagnosed in six patients from the capsule group and eight patients from the enteric tube group. All cases of rCDI were managed with oral vancomycin, ensuring a standardized regimen of 500 mg q.i.d. for at least five days prior to repeated FMT. All non-responders agreed to undergo a second FMT using the same or other administration method as their initial procedure. Following the repeated FMT, no further recurrences were observed within the eight-week follow-up period in any of the 14 patients. FMT cure rates are summarized in Fig. 3.5.1.1.

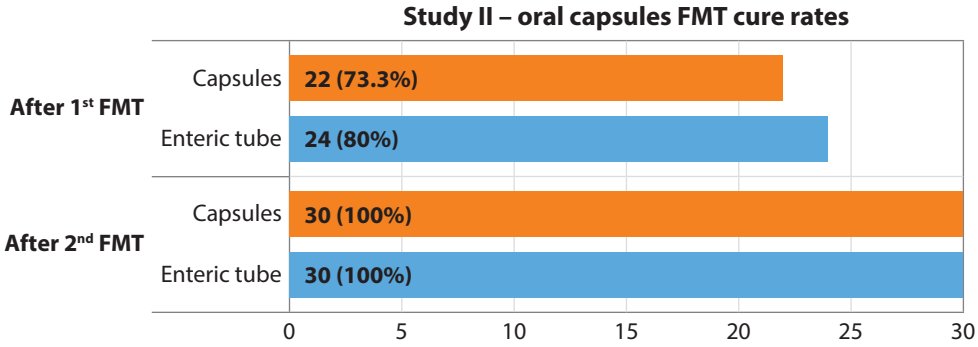


Fig. 3.5.1.1. *FMT cure rates after first and second FMT*

3.5.2. Early side effects and long-term follow-up

Consistent with findings from Study I, minor, self-limiting side effects were observed in both groups following FMT. The most commonly reported symptoms included mild nausea, abdominal discomfort, and increased bowel movements, all of which were resolved within a few hours and could be attributed to the underlying CDI rather than the FMT itself. No SAEs such as endoscopic complications, regurgitation, aspiration pneumonia, or fever, were recorded in either group. Among the 60 patients, 12 had IBD as a comorbidity; however, none experienced a disease flare shortly after FMT.

All participants were monitored for six months to assess potential late-onset complications. Throughout this follow-up period, no new diagnoses of oncologic, infectious, autoimmune, or other conditions potentially linked to FMT were observed. Additionally, no FMT-related deaths occurred within the Study II cohort.

4. DISCUSSION

The rising incidence of CDI, along with the growing number of recurrent and refractory cases, has prompted increased attention to novel therapeutic approaches such as FMT. Conventional antibiotic therapy offers only short-term resolution, with recurrence rates reaching approximately 20% after a first episode and up to 60% following multiple recurrences [33, 34]. Advances in our understanding of the human gut microbiome, particularly the concept of dysbiosis induced by external factors such as antibiotic use, have highlighted the potential of microbiome modulation in CDI management.

Although the complete pathophysiology of CDI is not yet fully understood, FMT has evolved from empirical intervention into a science-based, evidence-supported therapy that significantly reduces morbidity and mortality in patients with rCDI [135]. Both studies presented in this work demonstrated favorable FMT outcomes using two distinct GI delivery methods. In Studies I and II, FMT via enteric tube achieved a primary cure rate of 80% following single administration. These results are comparable with the protocol from the original study by van Nood et al. [19].

The efficacy of enteric tube-based FMT varies across published literature and meta-analyses. Smaller studies tend to report more variable outcomes, with cure rates ranging from 73.1% to 87.5% [180, 183, 186], likely influenced by limited sample sizes and potential selection bias. In a multicenter study conducted by a high-expertise stool bank, a primary cure rate of 89% was reported when FMT was administered via enteric tube [128]. Conversely, a meta-analysis by Ianiro et al. [156], which pooled data from 15 studies, found duodenal delivery to be effective in approximately 73% of patients with rCDI. The largest meta-analysis to date, conducted by Osman et al. [37] and including 899 patients who received FMT via upper GI routes, reported a primary cure rate of around 70%. The results from Studies I and II presented in this work are consistent with those from other published data, supporting the continued success of our FMT program and highlighting its effectiveness as a treatment option for rCDI.

Orally administered FMT capsules represent a less invasive and more patient-centered alternative to traditional FMT delivery methods. Currently, oral capsules, alongside colonoscopy, are among the recommended modalities for treating rCDI [14]. Reported efficacy following a single capsule-based FMT varies, with cure rates ranging from 70% to 89.5% [28, 227, 228]. Existing evidence indicates that a minority of patients require repeated FMT to achieve full resolution of CDI symptoms. Sequential administration of oral capsules has emerged as an effective strategy to enhance treatment outcomes.

Compared to other delivery routes, capsule-based FMT is simpler to perform and has been associated with improved cumulative cure rates [190, 229]. Meta-analyses examining the efficacy of oral capsule FMT report sustained cure rates between 71% and 92.2%, particularly when repeated FMT administrations are employed [37, 230, 231].

In Study II, the efficacy of frozen oral capsules was assessed, yielding a 73.3% primary cure rate after a single FMT. These findings align with previously published data; however, the highest success rates with oral capsules have been reported in studies utilizing sequential administration. The observed outcomes suggest that protocol optimization, particularly incorporating multiple FMT sessions – may be essential to fully realize the therapeutic potential of oral capsule FMT. In both Study I and Study II, repeated FMT significantly improved overall cure rates, supporting the need for future protocol adjustments to maximize efficacy.

Heterogeneous results across studies suggest that both procedural and patient-related factors may influence the success of FMT. Currently, the most consistently identified risk factor for FMT failure is the use of non-CDI-related antibiotics following the procedure [128, 179, 232, 233]. Additionally, multiple studies have reported that inpatient status is associated with an increased risk of rCDI after FMT [187, 188, 233, 234]. Earlier in the development of FMT, factors such as immunosuppression, IBD, inadequate bowel preparation, and advanced age were proposed as potential contributors to FMT failure. However, the evidence supporting these associations remains inconsistent and fragmented [187, 188, 233, 235, 236]. As larger studies have been conducted and more robust datasets analyzed, emerging evidence suggests that immunosuppression does not significantly compromise FMT efficacy [141, 142, 237]. Several studies comparing FMT outcomes between immunocompetent and immunosuppressed patients have found no significant differences in treatment success rates suggesting that immunosuppression alone should not be considered a contraindication to FMT in rCDI management [128, 150, 176, 179, 180, 184, 194, 237–240].

As FMT has been increasingly applied for rCDI in patients with IBD, growing evidence suggests that IBD is not associated with reduced FMT efficacy [151, 241, 242]. Several studies comparing outcomes between IBD patients and the general FMT population found no significant differences in cure rates, supporting the conclusion that FMT is an effective and recommended treatment option for rCDI in the IBD subgroup [128, 176, 181, 183, 243]. In Study I, a direct comparison between responders and non-responders was performed. No statistically significant differences were observed in age, gender distribution, number of previous CDI episodes, immunosuppressive therapy, or the prevalence of multimorbidity among the 60 patients. These

findings are consistent with existing literature and further support the conclusion that FMT is an effective treatment for rCDI across a range of clinical scenarios. It is important to emphasize that patients with CDI and their general practitioners (GPs) should be advised to follow rational antibiotic prescribing practices after FMT, as inappropriate antibiotic use remains the most significant modifiable risk factor for FMT failure. Other potential risk factors cannot either be modified or lack sufficient evidence to indicate a significant impact on treatment outcomes.

In Studies I and II, FMT was administered via enteric tube and oral capsules without prior bowel lavage. While there is evidence suggesting that bowel preparation may enhance FMT efficacy when using lower GI delivery methods and is recommended by current clinical guidelines [180, 232, 239]. However, the bowel preparation role in upper GI administration remains less clear. Recommendations supporting bowel cleansing prior to upper GI FMT are largely based on indirect evidence, primarily suggesting it may help eliminate residual vegetative *C. difficile* or remaining vancomycin [21, 244]. Omitting bowel preparation offers advantages in terms of patient comfort, particularly in elderly or polymorbid populations, where bowel cleansing can be burdensome or clinically challenging. The primary efficacy outcomes in both Study I and Study II were comparable to results reported in studies utilizing bowel preparation prior to upper GI FMT administration [19, 32, 245]. These findings highlight the need for larger, well-designed comparative studies to directly assess the impact of bowel preparation on FMT efficacy via upper GI routes.

The safety of FMT remains a primary concern for clinicians and regulatory authorities. FMT is based on the use of minimally processed, live donor feces containing a complex and diverse array of microorganisms, including bacteria, fungi, viruses, archaea, and yeasts [246]. Due to this biological complexity, standardization comparable to that used in pharmaceutical manufacturing is not feasible. Each donor's microbiota composition is inherently unique, and even individual samples from the same donor can vary significantly [247]. Despite these limitations, FMT has demonstrated substantial clinical benefit in the treatment of rCDI and refractory *C. difficile* infection, particularly in cases where standard antibiotic therapy fails to achieve sustained cure – making FMT a potentially life-saving intervention.

The primary safety concern is the potential transmission of enteric or blood-borne pathogens to recipients. To mitigate this risk, expert panels have established rigorous donor selection protocols, including comprehensive questionnaires and extensive screening of both blood and stool samples. These protocols have evolved in response to emerging threats such as the SARS-CoV-2 pandemic and increasing awareness of multidrug-resistant

organism (MDRO) transmission risks [21, 35, 165, 248]. All 120 patients included in Studies I and II received FMT material from thoroughly screened donors, with protocols adapted to reflect evolving evidence and safety recommendations. As a result, no infectious complications attributable to FMT were observed during short-term or long-term follow-up periods in either study, supporting the safety of the implemented donor screening framework.

Immunosuppressed individuals are considered a higher-risk population for FMT due to concerns about potential infectious complications. However, data from observational studies suggest that FMT is generally safe in patients with mild to moderate immunosuppression, with rates of SAEs comparable to those in immunocompetent populations [25–27, 249–251]. In contrast, FMT in severely immunosuppressed patients – such as those undergoing active cytotoxic chemotherapy, recipients of hematopoietic cell transplantation, individuals with neutropenia, severe primary immunodeficiencies, or untreated HIV infection – is not currently recommended due to insufficient safety data [145]. In both Study I and Study II, immunosuppressed patients with rCDI were included and underwent FMT via oral capsules or enteric tube delivery. No FMT-related SAEs were observed in this subgroup. It is important to note, however, that none of the included FMT recipients met the criteria for severe immunosuppression as defined above.

To date, the largest systematic review on FMT safety, conducted by Rapoport et al. [156] reported a SAE rate of 0.65%. Life-threatening complications such as aspiration pneumonia (0.27%), bacteremia or sepsis (0.19%), and bowel perforation (0.20%) were found to be exceedingly rare. The authors concluded that most SAEs were primarily associated with contaminated FMT products or improper administration practices, rather than the FMT procedure itself. Similarly, another large-scale study estimated a pooled SAE rate of 3.6%; however, upon detailed analysis, only 0.1% of events could be directly attributed to FMT [37]. In Study I, patients were followed for a median duration of 22 months (range: 1–55 months), while the Study II cohort was monitored for six months post-FMT. Across both studies, no FMT-related SAEs were observed, suggesting that rigorous donor screening and adherence to standardized FMT administration protocols may significantly reduce the risk of complications. Nonetheless, it is important to acknowledge that the relatively modest sample size may limit the generalizability of these findings. Continued data collection through the ongoing FMT program will help further elucidate the long-term safety profile of FMT.

4.1. Study limitations

Several limitations of the studies conducted should be acknowledged. In Study II, subgroup analyses involved relatively small sample sizes, which may affect the reliability and generalizability of the findings. Moreover, the Study I utilized fresh fecal material for FMT, which is no longer recommended, as frozen feces have been shown to offer comparable efficacy along with improved traceability. Consequently, following the emergence of updated guidelines, all subsequent FMTs were performed using frozen preparations.

Both studies were limited by fragmented and incomplete data on pre-FMT treatment and patient medical history, as the majority of FMT candidates were referred from regional hospitals. Collecting long-term follow-up data was challenging due to the frail condition of FMT recipients, which made follow-up assessments difficult and complicated the determination of causality for changes in clinical status. Additionally, neither study included a placebo or vancomycin monotherapy comparison group. Previous evidence suggests that vancomycin alone may achieve cure rates of up to 45% in rCDI populations [252]. However, comparative analyses of different FMT administration methods still provide valuable insights. Lastly, both studies used stool donations from multiple donors collected at different time points. It is well established that microbiota composition varies significantly between healthy individuals and can also fluctuate over time within the same donor. These variations may influence the microbial profile of each transplant and potentially affect clinical outcomes. Nonetheless, the consistent efficacy observed across both studies highlights the robustness of the FMT program, despite the inherent complexity and logistical challenges of managing a stool bank.

Future research would benefit from improved strategies for stratifying FMT patient inclusion, particularly for individuals with severe comorbidities and limited life expectancy. These patients may derive greater benefit from vancomycin therapy, as FMT carries potential risks and requires additional human resources, clinical expertise, and financial investment.

CONCLUSIONS

1. Fecal microbiota transplantation with fresh donor feces via enteric tube was effective for treating recurrent *Clostridioides difficile* infection, achieving an 80% cure rate after a single infusion. Sequential FMTs in non-responders increased the final cure rate to 100% within eight weeks.

2. Oral administration of frozen fecal capsules achieved a primary cure rate of 73.3%, thereby maintaining non-inferior therapeutic efficacy when compared to upper gastrointestinal fecal microbiota transplantation routes.

3. Oral frozen capsule and frozen feces enteric tube FMT methods showed no significant difference in efficacy ($P = 0.97$), with primary cure rates of 73.3% and 80%, respectively, for treating recurrent *Clostridioides difficile* infection.

4. Fecal microbiota transplantation via the upper gastrointestinal tract – whether administered through an enteric tube or oral frozen capsules – demonstrated an excellent safety profile. No serious or life-threatening complications, nor any new diagnoses potentially associated with fecal microbiota transplantation, were observed in either study group.

SANTRAUKA

ĮVADAS

Daugelis žmogaus organizmo vietų yra kolonizuotos įvairių mikroorganizmų, tačiau didžiausia jų populiacija randama virškinamajame kanale (VK) [1]. Šiuo metu jau įrodyta, kad žarnyno mikroorganizmai dalyvauja virškinant maistą, vykdant maisto medžiagų įsisavinimą, vitaminų gamybą, reguliuoja žarnyno imuninį atsaką ir moduliuoja žarnyne vykstančius uždegiminius procesus [2]. Žarnyno mikrobiotos sudėtį formuoja išoriniai arba aplinkos ir vidiniai, dar kitaip vadinami šeimininko, veiksniai. Pagal atliktų tyrimų duomenis reikšmingiausi šeimininko veiksniai yra skrandžio pH lygis, tulžies rūgšties kiekis, žarnyno motorikos ir imuninės sistemos aktyvumas. Išorinių faktorių gali būti žymiai daugiau, tačiau didžiausią įtaką daro mitybos įpročiai, asmeninė higiena, aplinkos tarša ir toksinai, fizinis aktyvumas, stresas ir miego režimas [1, 3]. Yra žinoma, kad nuolatinė žmogaus mikrobiotos sudėtis susiformuoja per pirmuosius 3–4 gyvenimo metus [4]. Žmogaus žarnyno mikrobiota yra pakankamai atspari trumpalaikiams trikdžiams ir turi mechanizmus, kurie leidžia pilnai atsistatyti po laikinų sudėties pokyčių. Tačiau vakarietiška mityba, maisto priedai, aplinkos tarša, paplitęs antibiotikų vartojimas gali turėti ilgalaikės neigiamos įtakos žmogaus žarnyno mikrobiotos įvairovei ir sudėčiai [5].

Šiuolaikinės medicinos klinikinėje praktikoje plataus spektro antibiotikų vartojimas yra plačiai paplitęs ir neatsiejamas nuo daugelio infekcinių ligų gydymo algoritmų. Būtent plataus poveikio antibiotikai sukelia reikšmingiausius žarnyno mikrobiotos pokyčius, o ilgalaikis jų vartojimas yra pagrindinis rizikos veiksnys žarnyno disbakteriozei atsirasti. Normalios žarnyno mikrobiotos įvairovės ir sudėties atsistatymas gali užtrukti mėnesius, o užsitęsusi disbakteriozė sukuria palankiams sąlygas patogeninei *Clostridioides difficile* (*C. difficile*) bakterijai [15, 16].

Pagrindinis ir dažniausiai pasireiškiantis *Clostridioides difficile* infekcijos (CDI) klinikinis požymis yra viduriavimas, kuris apibrėžiamas kaip tuštinimasis ≥ 3 kartus per dieną neformuotomis išmatomis, o infekciją padeda patvirtinti teigiamas *C. difficile* toksinų tyrimas išmatose [14, 17, 18]. Viduriavimas yra dažniausias, bet ne vienintelis CDI klinikinis požymis pagal kurią diagnozuojamas enterokolitas. Neretai pacientams pasireiškia pilvo skausmas, karščiavimas ir bendras negalavimas [68]. Svarbu paminėti, kad dalis CDI atvejų neturi tipiškos klinikinės eigos, o kai kuriais atvejais *C. difficile* toksinų tyrimai išmatose nebūna neinformatyvūs. Vienas iš alternatyvių infekcijos diagnozės nustatymo būdų – kolonoskopija. Endoskopinio tyrimu metu aptinkamas CDI būdingas vaizdas su pseudomembranomis, o galutinė

diagnozė patvirtinama storosios žarnos gleivinės biopsijomis su histopatologiniu tyrimu [83].

Pirmąjį CDI atvejį rekomenduojama gydyti *C. difficile* veikiančiais geriamais antibiotikais. Pagal naujausias ir klinikinėje praktikoje naudojamas gaires skiriamas 10 dienų gydymo kursas geriamu vankomicinu arba fidaksomicinu [88–90]. Tačiau nepaisant sėkmingo pirminio CDI gydymo iki 10–30 proc. pacientų patiria pasikartojančias *C. difficile* infekcijas (pCDI), o kiekvienas pCDI atvejis dar labiau padidina infekcijos recidyvo riziką [33, 34, 107, 109]. Taip pat svarbu paminėti, kad pCDI iki 33 proc. padidina pacientų mirtingumą palyginus su pacientais, kuriems liga nebus diagnozuota pakartotinai [110]. Pirmas infekcijos recidyvas gydomas pakartotiniu vankomicino ar fidaksomicino kursu, o anksčiau plačiai naudotas metronidazolas neberekomenduojamas pCDI gydymui dėl nepakankamo efektyvumo ir galimo neurotoksiškumo [107–109, 113, 114].

Pakartotiniai *C. difficile* enterokolitai ir dažni, ilgos trukmės antibiotikų kursai sumažina žarnyno mikrobiotos įvairovę, sukuria palankias sąlygas patogeninei bakterijai, todėl didėja pCDI rizika. Ieškant ilgalaikio sprendimo kaip atstatyti žarnyno eubiozę buvo atkreiptas dėmesys į išmatų mikrobiotos transplantaciją, kuri potencialiai galėtų išspręsti pagrindinę problemą – įgytą žarnyno disbakteriozę. Pirmosios užuominos apie išmatų mikrobiotos persodinimą randamos liaudies medicinos šaltiniuose dar IV amžiuje po kr. Tačiau detaliai aprašyti moksliniai įrodymai pirmą kartą publikuoti Beno Eisemano (Ben Eisman, angl.) 1958 metais, kuris sėkmingai gydė pseudomembraninį kolitą su donorinių išmatų klizmomis [119]. Kelis dešimtmečius šis gydymo metodas nebuvo plačiai naudojamas, tačiau XXI a. pradžioje pradėję eksponentiškai didėti pCDI atvejų skaičiai vėl atkreipė dėmesį į šią nestandartinę terapiją. 2008 m. buvo publikuota apie 100 pavienių žarnyno mikrobiotos transplantacijos (ŽMT) atvejų su daug žadančiu 90 proc. efektyvumu gydant pCDI [122]. 2013 metais Nyderlandų tyrėjai publikavo pirmąjį atsitiktinių imčių kontroliuojamą tyrimą, kuriame palyginimo vankomicino ir ŽMT efektyvumą gydant pCDI. Šioje studijoje buvo pasiektas 81 proc. gydymo efektyvumas po vienos donorinių išmatų infuzijos per enterinį zondą, o tuo tarpu vankomicino grupėje fiksuotas tik 31 proc. efektyvumas [19]. Vėliau sekusiuose tyrimuose ŽMT buvo lyginta su placebu, vankomicinu ir fidaksomicinu. Gydymo efektyvumas po vienos ŽMT procedūros svyravo nuo 65 proc. iki 92 proc., o pirminiai rezultatai rodė, kad pakartotinės ŽMT efektyvumą gydant pCDI padidina iki 90 proc. [19, 25, 26, 28].

Daugėjant įrodymų apie ŽMT potencialą gydant pCDI, jos pritaikymas klinikinėje praktikoje didėjo, tačiau atsirado pagrįstų abejonių dėl procedūros saugumo. 2019 metais publikuotose rekomendacijose pripažįstama, kad duomenys apie ŽMT saugumą nevienalyčiai, trūksta sisteminių, apibendrintų

duomenų [21]. Tačiau ekspertai pripažįsta, kad jau publikuotose tyrimuose šalutinių reiškinių dažnis yra mažas, dauguma jų susiję su nepavojingais VK simptomais, o ŽMT klinikinėje praktikoje labai svarbi ir dažnai gelstanti terapija gydanti pCDI [35, 136, 137].

Pasikartojančios ar standartiniam gydymui atsparios CDI gydymas klinikinėje praktikoje yra sudėtingas, nes sėkmingo antibakterinio gydymo poveikis trumpalaikis ir negarantuoja ilgalaikės ligos remisijos. Šiuo metu gerai žinoma, kad pakartotinių infekcijų rizika po pirmos CDI yra apie 20 proc. ir didėja iki 60 proc. po pasikartojančių infekcijos atvejų [33, 34]. ŽMT, kaip papildoma terapija greta gydymo antibiotikais, klinikine verte vis mažiau abejojama, tačiau ši procedūra išlieka eksperimentinė ir reikalaujanti papildomų tyrimų bei standartizacijos [14, 21–24, 35]. Šiuo metu mikrobiotos transplantacija prieinama tik akademiniams centrams su didele patirtimi atliekant žmogaus mikrobiomo tyrimus, todėl reikalingi standartizuoti, lengviau prieinami ŽMT metodai [36].

Mokslinio darbo naujumas ir aktualumas

Dabartinėje pCDI gydymo klinikinėje praktikoje dažniausiai naudojami apatinio VK ŽMT metodai. Didžioji dalis ŽMT atliekama kolonoskopijos metu, kai išmatų tirpalas sušvirkščiamas tiesiogiai į dešiniąsias storosios žarnos dalis [37]. Šioje disertacijoje tiriamas ŽMT gydymo efektyvumas naudojant alternatyvius viršutinio VK ŽMT būdus – enterinio zondo ir šaldytų kapsulių metodus. Antroje tyrimo dalyje buvo tiesiogiai lyginamos enterinio zondo ir kapsulių grupės pacientų charakteristikos ir palygintas šių metodų gydymo efektyvumas. Viršutinio VK metodų vystymas yra svarbus tobulint mažiau invazines ŽMT atlikimo strategijas, kurios būtinos gydant senyvus ir keliomis lėtinėmis ligomis sergančius pacientus.

Pastaruoju metu ŽMT pripažįstama kaip svarbi CDI gydymo dalis, tačiau išlieka nemažai neatsakytų klausimų dėl procedūros saugumo. Nepaisant didėjančių ŽMT apimčių trūksta duomenų apie ilgalaikį išmatų transplantacijos saugumą. Ypatingai svarbu įvertinti ar ŽMT nedidina infekcinių, metabolinių, onkologinių ar autoimuninių ligų rizikos. Šios disertacijos metu tiriamas ilgalaikis žarnyno mikrobiomo moduliacijos poveikis ŽMT recipientų sveikatai. Pirmojoje tyrimo dalyje analizuojami ilgo periodo saugumo rezultatai, o antroje dalyje nagrinėjamas abiejų viršutinio VK metodų – enterinio zondo ir geriamųjų kapsulių ilgalaikis saugumas.

Tikslas

Tyrimo tikslas yra įvertinti žarnyno mikrobiotos transplantacijos klinikinį efektyvumą ir ilgalaikį saugumą gydant pasikartojančią *Clostridioides difficile* infekciją skirtingais viršutinio virškinamojo kanalo mikrobiotos transplantacijos metodais.

Uždaviniai

1. Įvertinti žarnyno mikrobiotos transplantacijos šviežiomis donoro išmatomis efektyvumą gydant pasikartojančią *Clostridioides difficile* infekciją, kai procedūra atliekama enterinio zondo metodu.
2. Įvertinti žarnyno mikrobiotos transplantacijos efektyvumą gydant pasikartojančią *Clostridioides difficile* infekciją, kai procedūra atliekama šaldytomis geriamosiomis kapsulėmis.
3. Palyginti enterinio zondo ir šaldytų kapsulių žarnyno mikrobiotos transplantacijos metodų efektyvumą, gydant pasikartojančią *Clostridioides difficile* infekciją.
4. Įvertinti žarnyno mikrobiotos transplantacijos procedūrinį ir ilgalaikį saugumą, kai mikrobiotos transplantacija atliekama per viršutinį virškinimo kanalą.

Tiriamieji ir tyrimo metodai

Bioetikos leidimas. Tyrimai atlikti gavus Kauno regioninio biomedicininių tyrimų etikos komiteto leidimą (2011-03-08 protokolo Nr.: BE-2-10, 2018-06-05 protokolo Nr.: P2-BE-2-31/2018). Visi tiriamieji pasirašė sutikimo dalyvauti tyrime formas.

Tiriamieji. Į pirmąją tyrimo dalį buvo įtraukta 60 paeiliui gydytų pacientų, kuriems dėl pCDI buvo tikslinga ŽMT. Visiems 60 pacientų procedūra atlikta per enterinės mitybos zondą, kai tirpalas sulašinamas tiesiai į dvylikapirštę žarną. Tyrimo dalyviai iki ŽMT buvo gydomi Lietuvos sveikatos universiteto ligoninėje Kauno klinikos arba pervežti iš kitų ligoninių visoje Lietuvoje. ŽMT buvo atliekamos nuo 2015 iki 2019 metų Gastroenterologijos klinikoje arba kitame Kauno klinikų padalinyje dalyvaujant gastroenterologui. Pacientų klinikinė būklė po ŽMT buvo sekama Kauno klinikų gastroenterologų iki 2020 metų rugsėjo 1 dienos.

Į antrąją tyrimo dalį buvo įtraukta 30 pacientų, kuriems ŽMT buvo atlikta su geriamosiomis šaldytomis kapsulėmis. Visiems pacientams buvo diagnozuota pCDI ir tikslinga atlikti ŽMT infekcijos recidyvo rizikai sumažinti. Šių recipientų klinikiniai duomenys palyginti su 30 pacientų, kuriems ŽMT šaldytomis išmatomis atlikta per enterinės mitybos zondą. Visiems 60 pacientų ŽMT atlikta stacionare nuo 2017 iki 2021 metų Kauno klinikų gastroentero-

logijos skyriuje arba kitame padalinyje prižiūrinti gastroenterologui. Pacientų būklė procedūros metu ir šešis mėnesius po ŽMT buvo prižiūrimi Kauno klinikų gastroenterologų.

Recipientai. Abiejų tyrimo dalių pacientams buvo nustatytas antras arba vėlesnis CDI infekcijos epizodas. Pagrindinis įtraukimo kriterijus – viduriavimas (tuštinimasis ≥ 3 k/d. neformuotos išmatos) ir teigiamas *C. difficile* toksinų A ir B tyrimas išmatose. Kartu su viduriavimu galimi ir kiti CDI požymiai – pilvo skausmas, karščiavimas, padidėję kraujo uždegiminiai rodikliai (C-reaktyvinis baltymas, leukocitozė), tačiau tai nebuvo būtina įtraukimo sąlyga. Abiejų ŽMT metodų recipientams nebuvo atliekamas žarnyno paruošimas, nes ŽMT atliktos per viršutinį VK.

Recipientų paruošimas prieš žarnyno mikrobiotos transplantaciją. Paruošimas prieš numatomą ŽMT buvo standartizuotas ir taikytas abiejose studijose, visiems dalyviams. Prieš suplanuotą ŽMT taikytas standartinis CDI gydymas geriamais antibiotikais. Dauguma pacientų buvo gydyti 10 dienų geriamo vankomicino kursu. Dėl neprieinamo vankomicino rajoninėse ligoninėse dalis pacientų gydyti metronidazolu. Norint standartizuoti gydymą ir užtikrinti tinkamą bakterijų įsitvirtinimą visiems pacientams, buvo skiriamas bent penkių dienų 500 mg ×4 per burną vankomicino kursas. Remiantis Gastroenterologijos klinikos ŽMT protokolu ir tikintis sumažinti skrandžio rūgšties poveikį donorinei mikrobiotai visi recipientai gavo 40 mg geriamo omeprazolo prieš suplanuotą procedūrą. Vankomicino skyrimas buvo nutraukiamas 24–48 valandos iki numatytos išmatų transplantacijos.

Donorų atranka ir ištyrimas. Donorų atranka, jų sveikatos būklės įvertinimas, kraujo ir išmatų tyrimai buvo atliekami pagal publikuotas ekspertų gaires ir naujai atsiradusias rekomendacijas [19, 21, 35, 165, 225]. Abiejuose tyrimo dalyse buvo naudotos keturių su recipientais nesusijusių donorų išmatos. Kiekvienai ŽMT buvo naudojamos tik vieno donoro išmatos – abiejuose studijose multidonorinės ŽMT nebuvo atliekamos. Išmatų donorystė buvo neatlygintina, donorai nesusiję su sveikatos apsaugos sistema ir nepatenka į pažeidžiamų visuomenės grupių gretas. Visi donorai buvo vyrai, jaunesni nei 35 metai, kurių kūno masės indeksas nuo 18,5 iki 24,9 kg/m^2 ir apklausos metu nebuvo nustatytų jokių rizikos veiksnių, kurie detaliai aptariami gairėse [21]. Potencialiems donorams buvo atliekami detalūs kraujo ir išmatų tyrimai, kurie sumažina tikimybę infekcinių ligų perdavimui recipientui. Donorų ištyrimas kartojamas kas du mėnesius, kiekvienas donoras atliko daugybines išmatų donacijas skirtingu laiku.

Išmatų paruošimas. Pirmoje tyrimo dalyje buvo naudojamos šviežios donorinės išmatos. Išmatos buvo surenkamos ir transportuojamos specialiuose induose palaikant 4 °C temperatūrą. Išmatų paruošimas buvo atlieka-

mas ne vėliau nei šešios valandos po jų donacijos. Kiekvienai ŽMT procedūrai buvo naudojama mažiausiai 50 g donorinių išmatų. Išmatos skiedžiamos, homogenizuojamos. Šio proceso metu paruošiamas apie 500 ml tūrio donorinių išmatų tirpalas, kuris supilamas į saulei nepralaidų ir uždarą konteinerį, kuris ŽMT metu jungiamas prie enterinės mitybos zondo.

Antroje tyrimo dalyje kapsulių gamyboje ir enterinio zondo metoduose buvo naudojamos tik šaldytos donorinės išmatos. Protokolo pakeitimai atlikti atsižvelgiant į naujai publikuotus duomenis, kurie tvirtino, kad šaldytos išmatos nepraranda bakterijų įvairovės, bet palengvina išmatų paruošimo procesą ir padidina intervencijos saugumą [21].

Kapsulių gamyboje buvo naudojamos tik šaldytos išmatos. Užsaldytos išmatos buvo skiedžiamos, centrifuguojamos, kietoji dalis panaudojama kapsulių gamyboje. Viso tyrimo metu naudotos dvigubos, skrandžio rūgščiai atsparios, želatinos pagrindu pagamintos kapsulės, kurių tikslinė veikimo vieta – plonoji žarna. Iš mažiausiai 50 g donorinių išmatų buvo pagaminama 50 kapsulių, kurios laikomos –20 °C temperatūroje iki ŽMT.

Žarnyno mikrobiotos transplantacijos procedūra

Enterinio zondo metodas. Pacientams prieš ŽMT buvo atliekama fibrogastroduodenoskopija (EGDS). Endoskopijos metu į nusileidžiančiąją dvylikapirštės žarnos dalį įvedamas enterinės mitybos zondas. Paciento palatoje paruošta tirpalo talpa prijungiama prie enterinio zondo, tirpalas lėtai sulašinamas per zondą. Po sėkmingos transplantacijos enterinės mitybos zondas praplaunamas fiziologiniu tirpalu ir pašalinamas. Norint sumažinti aspiracijos riziką ŽMT metu visi pacientai gulėjo ant nugaros, pakeltu lovos galvūgaliu iki 45°. Po atliktos ŽMT pacientai iki šešių valandų stebėti stacionare.

Kapsulių metodas. Kiekvienas pacientas stacionare ŽMT atlikimo dieną per 2–3 valandas išgerdavo 50 šaldytų kapsulių su vandeniu. Norint sumažinti aspiracijos riziką pacientai lovoje privalėjo sėdėti pakeltu galvūgaliu iki 45°. Visi tiriamieji buvo palikti medicininio personalo stebėjimui mažiausiai šešias valandas.

Rezultatų vertinimas. Pirmoje ir antroje tyrimo dalyse ŽMT efektyvumas buvo vertinamas pagal klinikinį recipiento atsaką į gydymą. Pagrindinis, tarptautinių gairių, patvirtintas sėkmingos ŽMT požymis yra išnykęs viduriavimas [17, 23, 85, 106]. Ankstyvu infekcijos recidyvu buvo laikomi atvejai, kai per savaitę po ŽMT nebuvo stebėta klinikinio paciento pagerėjimo – išliko viduriavimas. Pacientai laikyti pasveikusiais, jeigu aštuonias savaites po atliktos ŽMT neatsinaujino viduriavimas. Kliniškai pasveikę recipientai nebuvo pakartotinai testuojami dėl *C. difficile* toksino išmatose, nes teigia-

mas toksinų tyrimas gali išlikti kelias savaites po sėkmingo ligos gydymo ir neturi klinikinės reikšmės [35].

Visi tiriamieji po atliktų ŽMT buvo stebimi ambulatorinių vizitų metu arba nuotolinių konsultacijų metu. Apklausos metu buvo vertinami duomenys apie pakartotinius CDI atvejus, naujai nustatytas diagnozes ir galimas komplikacijas.

REZULTATAI

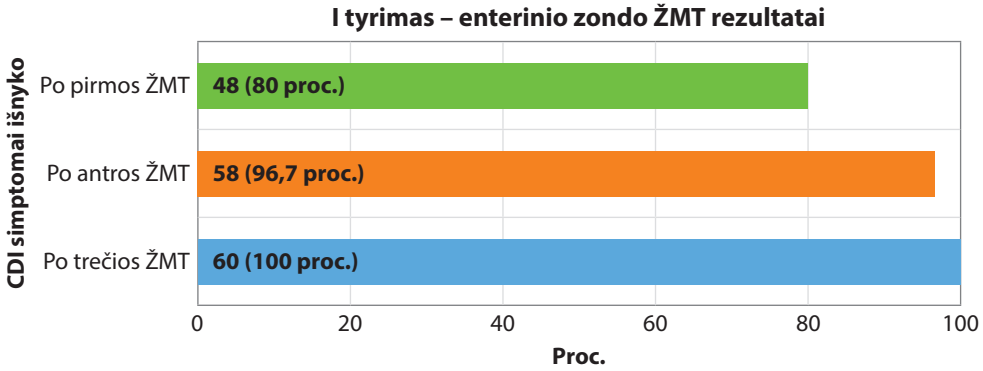
Pirmosios dalies rezultatai. Pirmoje tyrimo dalyje dalyvavo 60 pacientų, kuriems ŽMT atlikta per enterinės mitybos zondą dėl pCDI. Vidutinis pacientų amžius 72,5 metai (intervalas 32–99 metai). Pasiskirstymas pagal lytis buvo tolygus – 28 moterys (46,7 proc.) ir 32 vyrai (53,3 proc.). Vidutinis ankstesnių CDI recidyvų dažnis buvo 2,7 ± 1,3, intervale tarp vieno ir septynių kartų. Iš 60 tyrimo dalyvių devyni (15 proc.) naudojo imunosupresinius vaistus dėl gretutinių patologijų ir tęsė gydymą ŽMT metu ir po jos. Net 50 iš 60 (83,3 proc.) pacientų buvo diagnozuotas poliligotumas, kuris apibrėžiamas kaip dvi ar daugiau lėtinės ligos anamnezėje.

Rezultatai po pirmos žarnyno mikrobiotos transplantacijos. Apibendrinus rezultatus gauta, kad 48 iš 60 pacientų pasveiko po pirmos ŽMT, o tai atitinka 80 proc. pirminį gydymo efektyvumą. 12 pacientų stebėtas ligos recidyvas – per aštuonias savaites po atliktos ŽMT pasireiškė viduriavimas arba viduriavimas išliko iki vienos savaitės po atliktos išmatų transplantacijos.

Rezultatai po antros žarnyno mikrobiotos transplantacijos. Visi 12 pacientų, kuriems nustatytas CDI recidyvas po pirmos ŽMT buvo pakartotinai gydomi standartiniu 500 mg ×4 per burną vankomicino kursu. Visiems 12 pacientų ŽMT kartota laikantis pradinio protokolo, tuo pačiu enterinio zondo metodu. Po antros ŽMT 10 iš 12 recipientų aštuonias savaites po transplantacijos nebesikartojo viduriavimas. Apibendrinus rezultatus, po dviejų ŽMT pasveiko 58 iš 60 pacientų, o gydymo efektyvumas padidėjo iki 96,7 proc.

Rezultatai po trečios žarnyno mikrobiotos transplantacijos. Likusiems dviem pacientams buvo stebėtas infekcijos recidyvas per aštuonias savaites po antros ŽMT. Vienas iš pCDI pacientų turėjo keletą gretutinių ligų, kurios galėjo turėti įtakos ligos eigai. Iki pCDI buvo diagnozuotas 2-o tipo diabetas, 4 st. lėtinė inkstų liga, Parkinsono liga, hipotiroidizmas, lėtinė obstrukcinė plaučių liga ir išeminė širdies liga. Kitas pacientas po antros ŽMT buvo gydytas plataus spektro antibiotikais dėl odos infekcijos, o vėliau ir pneumonijos, kas galėjo turėti įtakos sumažėjusiam ŽMT efektyvumui. Abiem pacientams buvo atmestos kitos, su *C. difficile* nesusijusios, viduria-

vimo priežastys ir ŽMT pakartota per enterinės mitybos zondą po standartinio 10 dienų gydymo geriamu vankomicinu. Apibendrinti rezultatai pateikiami 1 pav.



1 pav. *Pirmojo tyrimo ŽMT rezultatai*

Pacientų duomenų palyginimas po pirmos žarnyno mikrobiotos transplantacijos. Atliktoje analizėje buvo palyginti 48 pacientų duomenys, kurie pasveiko po pirmos ŽMT su 12 pacientų duomenimis, kuriems prireikė pakartotinių intervencijų. Pagal atliktus skaičiavimus statistiškai reikšmingo skirtumo tarp grupių dalyvių pagal amžių (p = 0,124), lyčių pasiskirstymą (p = 0,697), ankstesnių CDI recidyvų dažnį (p = 0,804), imunosupresinių vaistų vartojimą (p = 0,365) ar poliligotumo paplitimą (p = 1) nebuvo. Apibendrinti duomenys pateikiami 1 lentelėje.

1 lentelė. *Pacientų po enterinio zondo ŽMT duomenų palyginimas*

	Kintamasis	Pasveiko (n = 48)	CDI atkrytis (n = 12)	p reikšmės
Amžius (metai)	Vidurkis ± SN	71,8 ± 12,9	61,7 ± 19,5	0,124
	Intervalas	[37–99]	[32–85]	
Pasiskirstymas pagal lytis	Moterys	27 (56,3 proc.)	6 (50 proc.)	0,697
	Vyrai	21 (43,6 proc.)	6 (50 proc.)	0,697
Ankstesnių CD dažnis	Vidurkis ± SN	2,6 ± 1,3	2,6 ± 1,3	
	Intervalas	[1–7]	[1–5]	0,804
Vartojami imunosupresiniai vaistai		6 (12,5 proc.)	3 (25 proc.)	0,365
Poliligotumas anamnezėje		40 (83,3 proc.)	10 (83,3 proc.)	1

Šalutiniai reiškiniai po žarnyno mikrobiotos transplantacijos. Lengvi, gyvybei nepavojingi šalutiniai reiškiniai po ŽMT yra neretai pasitaikantys ir neišvengiami. Dalis pacientų skundžiasi pykinimu, diskomfortu pilve, pagreitėjusia peristaltika, tačiau dauguma šių simptomų išnyksta per keletą valandų po transplantacijos. Šie simptomai nebuvo dokumentuojami, nes klinikinėje praktikoje sunkiai atskiriami nuo CDI sukeliamų simptomų ir neturi ilgalaikės reikšmės ar įtakos gydymo efektyvumui. Vienam iš pacientų stebėtas karščiavimas, tačiau tai neturėjo įtakos gydymo efektui, ligos recidyvo nestebėta po pirmos ŽMT. Su endoskopija ir enterinio zondo įvedimu susijusių komplikacijų tiriamosiose grupėse nestebėta.

Pirmos tyrimo dalies ilgalaikiai žarnyno mikrobiotos transplantacijos saugumo rezultatai. Vidutinė pacientų sekimo trukmė po ŽMT buvo 20 mėnesių (intervalas 1–55 mėnesiai). Svarbu paminėti, kad trys pacientai mirė ankstyvu periodu (aštuonios savaitės) po ŽMT. Atlikus medicininės dokumentacijos analizę tiesioginio ryšio tarp ŽMT ir mirčių nenustatyta, pacientai mirė dėl anksčiau diagnozuotų gretutinių patologijų. Praėjus trims savaitėms po atliktos ŽMT vienam recipientui diagnozuotas dinaminis žarnų nepraeinamumas. Po konservatyvaus gydymo stacionare pacientas pasveiko, CDI recidyvų šiam dalyviui nebuvo stebėta. Įvertinus medicininius duomenis, žarnų nepraeinamumo diagnozė tikėtina nesusijusi su mikrobiotos transplantacija.

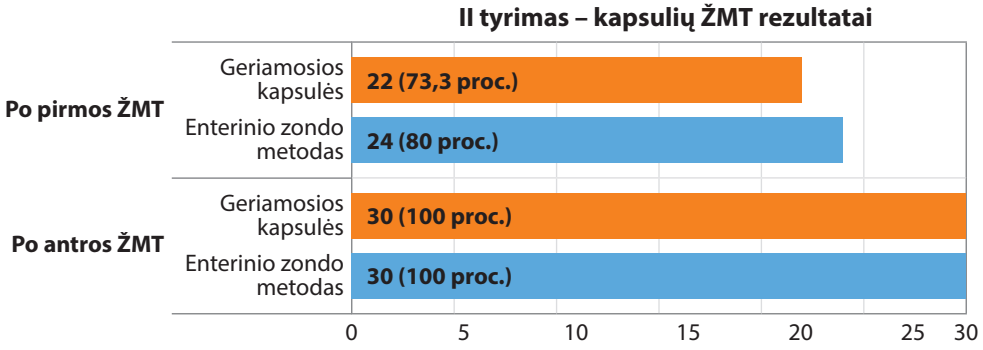
Antros tyrimo dalies rezultatai. Antroje tyrimo dalyje 30 pacientų atlikta ŽMT šaldytomis geriamosiomis kapsulėmis, o šių pacientų duomenys palyginti su 30 pacientų, kuriems ŽMT šaldytomis išmatomis atlikta per enterinį zondą. Kapsulių grupės pacientų amžiaus vidurkis 66,03 ± 20,69 metai, o enterinio zondo grupės – 66,23 ± 17,89 metai. Abiejuose tiriamųjų grupėse buvo saikiai daugiau moterų – 16 (53 proc.) kapsulių ir 18 (60 proc.) enterinio zondo grupėje. Daugiau nei pusei pacientų buvo nustatytas poliligotumas – 17 (56,7 proc.) ir 19 (63 proc.) atitinkamai kapsulių ir zondo grupėse. Abiejose tiriamosiose grupėse buvo po šešis pacientus, kuriems iki pCDI diagnozės jau buvo nustatytos ir gydomos uždegiminės žarnų ligos (UŽL). Kapsulių ir zondo grupėse buvo vienodas skaičius imunosupresinių ligonių – po aštuonis. Palyginus šių grupių pacientų duomenis nebuvo pastebėta statistiškai reikšmingo skirtumo tarp amžiaus ($p = 0,97$), lyčių pasiskirstymo (vyrų $p = 0,82$, moterų $p = 0,66$), poliligotumo ($p = 0,79$), sergamumo UŽL ($p = 1$) ar imunosupresinės būklės ($p = 1$). Palyginimo rezultatai pateikiami 2 lentelėje.

2 lentelė. *ŽMT kapsulėmis ir enteriniu zondu pacientų duomenų palyginimas*

Kintamasis	ŽMT kapsulėmis (n = 30)	ŽMT enteriniu zondu (n = 30)	p reikšmė
Amžius, vidurkis ± SN	66,03 ± 20,69	66,23 ± 17,89	0,97
Vyrai	14 (47 proc.)	12 (40 proc.)	0,82
Moterys	16 (53 proc.)	18 (60 proc.)	0,66
Poliligotumas	17 (56,7 proc.)	19 (63 proc.)	0,79
Uždegiminės žarnų ligos	6 (20 proc.)	6 (20 proc.)	1
Opinis kolitas	4 (13,33 proc.)	5 (16,67 proc.)	1
Krono liga	2 (6,67 proc.)	1 (3,33 proc.)	1
Imunosupresinė terapija anamnezėje	8* (26,67 proc.)	8** (26,67 proc.)	1
Gliukokortikoidai	5 (16,67 proc.)	8 (26,67 proc.)	0,55
Kiti imunosupresantai	2 (6,67 proc.)	5 (16,67 proc.)	0,43
Biologinė terapija	4 (13,33 proc.)	0 (0 proc.)	0,12

* 3 pacientai gavo daugiau nei 1 imunosupresinį vaistą; ** 5 pacientai gavo daugiau nei 1 imunosupresinį vaistą.

Žarnyno mikrobiotos transplantacijos gydymo efektyvumas. Kapsulių grupėje 22 iš 30 pacientų pasveiko po pirmos ŽMT, todėl pirminis efektyvumas šioje grupėje yra 73,3 proc. Enterinio zondo grupėje 24 iš 30 pacientų viduriavimas nebesikartojo aštuonias savaites po pirmos procedūros, buvo pasiektas 80 proc. pirminis efektyvumas. Reikšmingo skirtumo tarp skirtingų gydymo metodų efektyvumo nenustatyta (p = 0,97). Aštuoniems kapsulių grupėje ir šešiems pacientams enterinio zondo grupėje pasikartojo viduriavimas per aštuonias savaites po pirmos ŽMT. Visiems patyrusiems CDI recidyvą buvo taikomas pakartotinis standartinis gydymas geriamu vankomicinu iki 10 dienų, atlikus pilną paruošimą pakartota ŽMT. Po antros transplantacijos aštuonių savaičių periode visi 14 pacientų išliko be CDI būdingų simptomų. Apibendrinti rezultatai pateikiami 2 pav.



2 pav. *Efektyvumo rezultatai ŽMT atlikus kapsulėmis*

Šalutiniai reiškiniai po žarnyno mikrobiotos transplantacijos. Antros studijos dalies pacientai buvo sekami iki šešių mėnesių po atliktos ŽMT. Ankstyvi, lengvi šalutiniai reiškiniai sutampa su pirmos dalies rezultatais – dalis pacientų juto lengvą pykinimą, diskomfortą pilve, pagreitėjusią peristaltiką. Daugumą simptomų išnyko per kelias valandas, reikšmingos įtakos gydymo eigai neturėjo. Šioje tyrimo dalyje nebuvo pastebėta sunkių šalutinių reiškinių – endoskopinių komplikacijų, pneumonijos, aspiracijos, karščiavimo ar kitų grėsmingų būklių. Iš 60 dalyvių net 12 turėjo anksčiau diagnozuotas UŽL. Literatūroje aprašomų retų UŽL paūmėjimų po atliktos ŽMT nebuvo užfiksuota. Ilguoju pacientų sekimo periodu naujų, su ŽMT susijusių, diagnozių, būklių ar mirčių nenustatyta.

IŠVADOS

1. Žarnyno mikrobiotos transplantacija šviežiomis išmatomis per enterinį zondą yra efektyvus metodas gydant pasikartojančią *Clostridioides difficile* infekciją. Efektyvumas po procedūros siekia 80 proc. o, atliekant pakartotines procedūras buvo pasiektas 100 proc. gydymo efektyvumas.
2. Po pirmos žarnyno mikrobiotos transplantacijos kapsulėmis buvo pasiektas 73,3 proc. *Clostridioides difficile* infekcijos gydymo efektyvumas ir savo efektyvumu nenusileidžia kitiems viršutinio virškinamojo kanalo žarnyno mikrobiotos transplantacijos būdams.
3. Enterinio zondo ir kapsulių žarnyno mikrobiotos transplantacijos pirminis efektyvumas gydant pasikartojančią *Clostridioides difficile* infekciją tarpusavyje reikšmingai nesiskyrė ($p = 0,97$) ir atitinkamai yra 80 proc. ir 73,3 proc.
4. Žarnyno mikrobiotos transplantacija per viršutinį virškinimo kanalą yra saugus gydymo metodas sergantiems pasikartojančia *Clostridioides difficile* infekcija. Tyrimo metu nebuvo stebėta sunkių ar gyvybei grėsmingų šalutinių reiškinių, o atliekant ilgalaikį pacientų stebėjimą nebuvo fiksuota naujų su žarnyno mikrobiotos transplantacijos susijusių patologijų.

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LIST OF PUBLICATIONS

1. Urbonas T, Ianiro G, Gedgaudas R, Sabanas P, Urba M, Kiudelis V, Kiudelis G, Petkevicius V, Vitkauskiene A, Cammarota G, Gasbarrini A, Kupcinskas J. Fecal microbiome transplantation for recurrent *Clostridioides difficile* infection: treatment efficacy, short and long-term follow-up results from consecutive case series. 2021. Journal of Gastrointestinal and Liver Diseases. 2021 Dec 21;30(4):470–476. doi: 10.15403/jgld-3800. PMID: 34752587.
2. Urbonas T, Petrauskas D, Kiudelis V, Jonaitis L, Skieceviciene J Gedgaudas R, Kiudeliene E, Valantiene I, Zykus R, Varkalaite G, Inciuraite R, Trapenske E, Kulokiene U, Jonaitis P, Ramonaite R, Velickiene J, Zvirbliene A, Morkunas E, Kuliaviene I, Sumskiene J, Adamonis K, Macas A, Kupcinskiene K, Lukosiene L, Janciauskas D, Poskiene L, Vitkauskiene A, Ianiro G, Gasbarrini A, Kiudelis G, Kupcinskas J. 2025. Original article: Fecal microbiome transplantation for recurrent CDI: treatment efficacy and safety with oral capsules. Journal of Gastrointestinal and Liver Diseases, 34(2), 1–6. https://doi.org/10.15403/jgld-5990.

LIST OF CONFERENCES

1. Bioateitis: gamtos ir gyvybės mokslų perspektyvos, 2021, Kaunas, Lithuania. Oral presentation "Žarnyno mikrobiotos transplantacija gydant pasikartojančią *C. difficile* infekciją: efektyvumas, ankstyvieji ir vėlyvieji pacientų sekimo rezultatai".
2. EHMSG 2021. 2021, virtual. Poster presentation "Fecal microbiome transplantation for recurrent CDI treatment: efficacy, short and long term follow up results from consecutive case series".
3. The International Scientific Conference on Medicine, 2021, Ryga. Oral presentation. "Long-term follow-up after fecal microbiome transplantation: results from consecutive case series".
4. EHMSG 2022, Glasgow, UK. Poster presentation "Fecal Microbiome Transplantation for Recurrent CDI: Treatment Efficacy and Safety with Oral Capsules".
5. 17th Congress of ECCO European Crohn's and Colitis Organisation Inflammatory Bowel Diseases 2022. 2022 virtual conference. Poster presentation "Fecal Microbiome Transplantation for Recurrent *C. difficile* Colitis: Treatment Efficacy, Short and Long term Follow up Results from Consecutive Case Series".
6. „Bioateitis: gamtos ir gyvybės mokslų perspektyvos", 2023, Vilnius. Oral presentation: "Žarnyno mikrobiotos transplantacija geriamomis kapsulėmis pasikartojančios *C. difficile* infekcijos gydymui: efektyvumo ir saugumo rezultatai". First place winner for the best oral presentation.
7. International Health Sciences Conference for All 2024. Kaunas. Oral presentation "Fecal microbiome transplantation for recurrent CDI: treatment efficacy and safety with oral capsules".

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