UNIVERSITATEA LUCIAN BLAGA DIN SIBIU

Şcoala doctorală interdisciplinară

Domeniul de doctorat: Medicină

TEZĂ DE DOCTORAT

PREVENȚIA RECURENȚELOR INFECȚIOASE CU CLOSTRIDIUM DIFFICILE - ROLUL TRANSPLANTULUI DE MATERII FECALE

doctorand:

IULIAN-DANIEL POPA

Conducător Doctorat:

Prof. univ. dr. MANUELA MIHALACHE

SIBIU 2022

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The infection with *Clostridium difficile* (CDI) has become, for the past years, an actual social problem due to the increase of its incidence and severity. *Clostridium difficile* (CD) is an anaerobic, spore-forming, Gram-positive bacteria, the major etiological factor of antibiotic-associated colitis;it represents the most common cause of diarrheic disease associated to public health services, accountable for an important morbidity and mortality.

At least two prerequisites are necessary for the occurrence of CDI: alteration of normal intestinal flora determining a decrease in resistance against colonization with Clostridium difficile and, acquisition of the organism from an exogenous source. Other factors are represented by the host's susceptibility, virulence of the strains, or nature and period of exposure to antibiotics.
The infection's transmission mechanism is orofecal, the excreting individual (whether healthy or sick) represents the source of infection, with more than 400 strains of Clostridium difficile in existence but, only toxin producing strains lead to the occurrence of the disorder.

By far, the most important risk factor is the use of antibiotics in the period foregoing onset, even in the form of a single prophylactic dose, almost any antibiotic can lead to the occurrence of the infection. If, certain risk factors cannot be intervened upon (age), there are attempts to

implement guides for the well-balanced use of antibiotic therapy and specific epidemiology measures for infected patients who are hospitalized nevertheless, the number of infections has not decreased. Other risk factors are also incriminated: anti-secretory medication, co-morbidities, immunosuppressed state.

CDI should be suspected in all patients with nosocomial diarrhoea, patients with unexplainable diarrhoea and new onset of ≥ 3 poorly formed stools/24 hours (types 5-7 on the Bristol scale), post antibiotic diarrhoea with community origin, elderly patients, those who were administered antibiotics, immunosuppressors or gastric antisecretory medication, those who don't belong to an acute diarrhoea focal group within the community are the ones representing the target population to be tested.

CDI becomes manifest only in the case of toxins' production as non toxin generating forms will not lead to diarrheic disease therefore, diagnosis relies on identifying toxins from either faeces, or from cultures of Clostridium difficile.

CDI diagnosis is a challenge as there is not one perfect lab test or a universal reference test to perform. There was a dispute relating to which of the reference tests, cell cytotoxicity neutralisation assay (CCNA) or toxigenic culture(TC) should be considered as the gold standard for detecting the infection but, each of them has strong points as well as limitation so, due to these imperfections of tests, a combination of tests was advanced in order to improve results.
The European Society of Clinical Microbiology and Infectious Disease (ESCMID) recommends the use of a diagnosis algorithm during several stages in order to increase diagnosis accuracy, the reasoning behind it being that of using a test with sensitivity as a first stage and a high specificity one subsequently. ESCMID recommends testing GDH (NAD-specific glutamate dehydrogenase) or NAAT (Nucleic acid amplification tests) as initial diagnosis steps. The high sensitivity of these tests confers then a very good negative predictive value therefore, a negative result is most likely an indicator of ICD absence. During the second diagnosis step, should the first test be positive, it is recommended to perform A and B toxins tests with a very high specificity granting them a high positive predictive value therefore, should the second test be positive, the presence of the infection is confirmed. In the event of initial positive tests and a negative one in the second step, it is believed that the result is ambiguous and the performance of toxicogenic cultures is recommended. An alternative algorithm recommended by ESCMID is to

concurrently analyze GDH and the present of toxins as a first step and, in the event of ambiguous results, to subsequently perform TC or NAAT testing. Lower digestive tract endoscopy is a very useful method for patients with symptoms suggesting ICD and negative test or in cases where the attainment of a sample for testing is not possible, as in the case of intestinal occlusion, nevertheless this is not routinely used for patients with typical symptoms and positive lab tests.

Recommendations for Clostridium difficile infection treatments have evolved and developed throughout time. In 2018, recommendations for first line treatment were vancomicin or fidaxomicin for 10 days. Concurrently with specific treatment methods, other additional treatment methods include: interruption of useless antimicrobial treatments, correction of hydroelectric balances, avoidance of motility inhibiting medication, and revision of proton pump inhibitors medication.

Although the great majority of ICD patients have a favourable response to specific antibiotic drug therapy, a significant percentage with present one or several relapses. And the risk of relapse increases with the increase in numbers of infectious episodes. After a first CDI episode, 11-25% patients will relapse within the first 30 days since the completion of therapy . After the first relapse episode, up to 46.2% of patients will have a second episode of relapse . Moreover, the risk of recurrence continues to rise, reaching 50–60% at the third episode.

These recurrences generate additional costs for the medical system, morbidity, and the risk of fatalities.

Despite having been empirically used for several centuries for the treatment of certain forms of diarrhoea, the first documented use of a human fecal microbiota suspension for the treatment of severe forms of diarrhoea dates back to 4th century China, fecal microbiota transplant (FMT) stands out as an extremely effective treatment method for patients with several recurrences or with severe forms of Clostridium difficile infections. There still are questions about the long-term safety profile, but severe immediate complications have been seldom reported. Currently, the FMT is included in the European treatment guidelines of CDI, for multiple recurrences , mild or severe rCDI and refractory CDI , while in the American treatment guideline of CDI, after the second or subsequent rCDI

Work hypothesis

The role of FMT in the treatment of a first infectious episode with Clostridium difficile is not yet determined as the existing data is insufficient at this time in order to recommend FMT from the first infectious episode, regardless of whether it has a mild form or a severe/complicated one, a small number of patients having been reported to date.

The main objective of this study was to determine whether the risk of post FMT recurrence. FMT performed after a complete 10 day antibiotic treatment, and the disappearance of symptoms, in the case of a Clostridium difficile severe primary infection. The secondary goals were the overall rate of success (primary and secondary) of FMT regardless of the number of infectious episodes, the rate of success of FMT after a severe form, identification of post FMT recurrence risk factors, and determination of post FMT recurrence risks after a second or third severe ICD episode.

Study Design

This is a retrospective study , we analyzed the clinical characteristics and the outcomes for 96 CDI patients with FMT, during January 2015 to July 2019 period. The research has been conducted in Sibiu County Clinical Emergency (Gastroenterology and Infection Disease Departments) and Sibiu Polisano Clinic (Gastroenterology Department), Romania and all the FMT procedures were undertaken in these two medical institutions in the county.

Data Analysis

Data is presented as percentages, medians. The potential risk factors for the outcomes consisted of age (computed as numerical variable in years) and the following as categorical variables: 1)gender, 2)comorbidities categories(malignant neoplasm, diabetes mellitus, hematological diseases, cardiovascular diseases, chronic digestive diseases, renal insufficiency), 3) clinical manifestations and paraclinical features (leukocytes , body temperature , serum creatinine , signs of septic shock , pseudo membranous colitis at endoscopy), 4) antibiotherapy or gastric acid suppression medication prior to CDI, antibiotherapy classes (Aminopenicillins ± beta lactamase−inhibitor, Cephalosporins, Carbapenems, fluoroquinolones and Clindamycin). Both the primary and the secondary outcomes were interpreted as categorical variables: i) the

recurrence post-FMT procedure after a primary severe CDI episode; ii) the primary overall FMT success; iii) the FMT success after a severe form or a non-severe form, iii) the recurrence risk for the post FMT procedure employed after a second and a third severe CDI episode, iv) the secondary overall success rate, v) the recurrence post FMT after a non-severe CDI.

We applied Fisher exact test for the categorical variables and Mann–Whitney test for continuous variables, respectively . The p value <0.05 was considered statistically significant. However, we considered the "trending toward clinical studies" to be clinically relevant and appropriate in our small sample retrospective study using the relaxed value of the α level to 0.10

Results

This study included 96 patients who underwent a fecal microbiota transplant throughout the development of the study (January 2015 - July 2019), 11 of them have been subjected to a second FMT.

Demographic and clinical characteristics of the patients

Number of patients	96
Age	
Median, years (range)	68.5 (20–89)
20-39 years, *n*(%)	15(15.6)
40-59 years, *n*(%)	13(13.5)
60-79 years, *n*(%)	56(58.3)
80-90 years, *n*(%)	12(12.5)
> 65 years, *n*(%)	58 (60.4)
Gender	
Female, *n* (%)	67 (69.8)
Male, *n* (%)	29 (30.2)
Comorbidities	
No, *n* (%)	16 (16.7)
Malignant neoplasm, *n* (%)	15(15.6)
Diabetes mellitus, *n* (%)	13(13.5)
Hematologic Diseases, *n* (%)	11(11.5)
Cardiovascular diseases, *n* (%)	58(60.4)
Chronic digestive diseases , *n* (%)	38(39.6)
Renal insufficiency, *n* (%)	14(14.6)
≥ 2, *n* (%)	46(47.9)
Antibiotics use prior to CDI	
No, *n* (%)	30(31.3)
Aminopenicillins±beta lactamase−inhibitor, *n* (%)	14(14.6)
Cephalosporins, *n* (%)	41(42.7)

Fluorochinolone,*n* (%)	27(28.1)
Carbapenems,*n* (%)	6(6.3)
Clindamycin,*n* (%)	1(1)
Associations,*n* (%)	23(24)
Acid-suppression medications	
No, *n* (%)	65(67.7)
Yes, *n* (%)	31(32.3)

85 patients who were subjected to a first fecal microbiota transplant have not demonstrated ICD relapse, represented a primary overall rate of success of 88.5% regardless of severity while 11 patients (11.5%) with a first fecal microbiota transplant demonstrated ICD relapse.

OF the 96 patients with FMT, for 25 patients (26%), the fecal microbiota transplant was performed during the first infectious episode, for 52 (54%) the transplant was performed during the second infectious episode, and for 19 patients (20%), the transplant was performed during their third infectious episode.

The overall FMT rate of success (including both non-severe and severe forms) per infectious episode was 92% for the first ICD episode, 88.4% for the second ICD episode, respectively 84.2 % for the third ICD episode.

1 patient suffering from a severe form during the second infectious episode died from causes not related to the fecal microbiota transplant.

From the 11 patients with relapse after the fecal microbiota transplant, for the patients, the transplant was performed after the first infectious episode therefore an 8% rate of post FMT relapse, for 6 patients transplant was performed after the second infectious episode representing a post FMT relapse rate of 11.5% and, for 3 patients, the transplant was performed after the third infectious episode indicating a post FMT relapse rate of 15.8%.

From these 11 patients, 9 showed relapse after a severe form of the disease (an initial rate of success after FMT in severe forms 87.5%, 1 patient with severe form wherefore FMT was performed after the first infectious episode representing a rate of relapse of 5.26%, 5 patients with severe form relapsed after FMT was performed following the second infectious episode indicating a relapse rate of 15.15% and all 3 patients relapsing after FMT was performed

following the third infectious episode had a severe form of the disease indicating a relapse rate of 27.3%.

The analysis of the recurrence risk based on the number of the infectious episode after which FMT was performed on patients with a severe form revealed a risk of relapse when FMT was performed upon the 2nd infectious episode ($p = 0.009$) or 3rd infectious episode ($p = 0.000$) but this risk was absent when FMT was performed after the 1st infectious episode ($p = 0.279$).

Among patients whereon FMT was performed for non-severe ICD, 1 patient (16.6%) relapsed after FMT performed for primary ICD and another patient (5.26% of the total) relapsed after FMT performed upon the second ICD episode resulting in a primary rate of success of 93.94%.

Patients with a second FMT, with both severe and non-severe forms have not showed another ICD relapse throughout the period of follow-up, resulting a secondary rate of success of 100%.

The risk of developing severe forms of ICD is significant in the case of patients with cardiovascular disease, with previous use of carbapenems or gastric antacid medication.

Age, the presence of comorbidities, use of antibiotics or gastric antisecretory medication have not been accounted as risk factors of post FMT relapse.

This paper also present a case study, the first communication in medical literature, the case of a patient with multiple ICD relapses, on rectal stump, after colectomy for a toxic megacolon induced by Clostridium difficile infection and symptomatic Clostridium difficile vaginal infection successfully treated with serial fecal mini-transplants and metronidazole ovules.

Conclusions

1. Metronidazole and vancomycin combination was the most used method for primary CDI treatment , but also for second and third CDI episode, and for severe CDI.
2. The risk of developing severe forms of ICD is significant in the case of patients with cardiovascular disease, with previous use of carbapems or gastric antacid medication.
3. The overall primary rate of success of FMT, including both severe and non-severe forms, was 88.5%.

4. The overall FMT rate of success was 92% for the first ICD episode, 88.4% for the second ICD episode, respectively 84.2 % for the third ICD episode.
5. Age, the presence of comorbidities, use of antibiotics or gastric antisecretory medication have not been accounted as risk factors of post FMT relapse.
6. Tygecicline was the most used antibiotic for CDI relapses treatment after FMT
7. The primary rate of success for non-severe forms was 93.94%.
8. Severe forms with 1 severity factor are not a post FMT risk factor but severe forms with ≥2 severity factors and the presence of leukocytosis are post FMT relapse risk factors.
9. The primary rate of success for severe forms was 85.7%.
10. The present study brings forth new data which supports the beneficial role of FMT in primary severe ICD, with post FMT rates of relapse in primary severe ICD being more reduced than rates after therapy with conventional medication.
11. There is an increase in the post FMT rate of relapse along with the number of the ICD episode.
12. The overall secondary rate of success of FMT was 100%.

Originality and innovative contributions of the thesis

Infections with Clostridium difficile represent a current public health issue in both Romania and the whole world due to the increase of its occurrence and severity. In the treatment of recurrent ICD forms, the use of fecal microbiota transplant has become regular. Specific data about FMT usefulness in Romania as ICED treatment is missing as this study was the first to report FMT rates of success, risk factors for post FMT relapse, risks of relapse based on the number of the episode after which FMT was carried-out.

At present times, FMT is included in the European Guide for ICD treatment, in the event of multiple recurrences, mild or severe cases of rICD nevertheless, the role of FMT in the treatment of the first ICD episode is yet to be determined as the data existing at this time is insufficient so as to recommend FMT ever since the first ICD episode, regardless of whether it is a mild or severe form of the disease. The present study includes the greatest number of patients subjected to FMT for primary ICD in severe form, bringing evidence in favour of FMT use for non-

recurrent ICD patients with severe forms, with lesser relapse rates compared to the rates of relapse following medication therapy.

This paper is the first to compare post FMT relapse risks depending on the episode after which FMT was performed, indicating a lesser risk of relapse for primary severe forms of ICD and also that, the risk of relapse increases along with the number of the ICD episode, data in favour of performing FMT as soon as possible in the event of severe forms.

ICD may manifest also through severe/complicated forms, toxic megacolon, perforations, and death. We achieved the first medical communication of an FMT carried-out successfully on a rectal blunt for multiple ICD, proving that serial administration, with the greatest volume tolerated by the patient is an effective method of treatment in the case of patients with post colectomy rectal blunts or refractory pouchitis for antibiotic treatment.