DANIEL ANTÔNIO DE ALBUQUERQUE TERRA

IMPLANTAÇÃO DO CENTRO DE TRANSPLANTE DE MICROBIOTA FECAL DO INSTITUTO ALFA DE GASTROENTEROLOGIA DO HOSPITAL DAS CLÍNICAS DA UFMG E ANÁLISE DOS PRIMEIROS RESULTADOS EM PACIENTES COM INFECÇÃO RECORRENTE OU REFRATÁRIA PELO *CLOSTRIDIOIDES DIFFICILE*

Universidade Federal de Minas Gerais

Programa de Pós-Graduação em Ciências Aplicadas à Saúde do Adulto

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Orientador: Prof. Dr. Luiz Gonzaga Vaz Coelho.
Co-orientador: Prof. Dr. Eduardo Garcia Vilela.

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IMPLANTAÇÃO DO CENTRO DE TRANSPLANTE DE MICROBIOTA FECAL DO INSTITUTO ALFA DE GASTROENTEROLOGIA DO HOSPITAL DAS CLÍNICAS DA UFMG E ANÁLISE DOS PRIMEIROS RESULTADOS EM PACIENTES COM INFECÇÃO RECORRENTE OU REFRATÁRIA PELO CLOSTRIDIOIDES DIFFICILE

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"Quem elegeu a busca, não pode recusar a travessia."

Alfredo Bosi

LISTA DE ABREVIATURAS E SIGLAS

AE - Adverse events
ACG - Colégio Americano de Gastroenterologia
AGA - Associação Americana da Gastroenterologia
ALT - Alanino aminotransferase
ASGE - Sociedade Americana de Endoscopia Gastrointestinal
AST - Aspartato aminotransferase
BMT - Bone marrow transplantation
CAAE - Certificado de apresentação de apreciação ética
CDI - *Clostridioides difficile* infection
CEP - Comitê de ética em pesquisa
CONEP - Comissão Nacional de Ética em Pesquisa
CMV - Citomegalovírus
CNS - Conselho Nacional de Segurança
CTMF - Centro de transplante de microbiota fecal
DII - Doença inflamatória intestinal
DNA - Ácido desoxirribonucleico
EA - Eventos adversos.
EBV - Epstein-Barr vírus
EBSERH - Empresa Brasileira de Serviços Hospitalares
ECPE - Escherichia coli enteropatogênica
ECTS - Escherichia coli produtora de toxina Shiga
ESBL - Betalactamase de espectro expandido
FDA - *Food and Drug Administration*
FMT - Fecal microbiota transplantation
GDH - Glutamato desidrogenase
GGT - Gama glutamil transpeptidase
GVHD - *Graft-versus-host disease*
HC-UFMG - Hospital das Clínicas da Universidade Federal de Minas Gerais
HDL - *High density lipoprotein*
HIV - Vírus da imunodeficiência humana

HTLV - Vírus T-linfotrópico humano

IAG - Instituto Alfa de Gastroenterologia

IBD - Inflammatory bowel disease

IBP - Inibidor de bomba de prótons

IC - Informed consent

ICD - Infecção pelo *Clostridioides difficile*

IDSA - Sociedade Americana de Doenças Infecciosas

IgG - Imunoglobulina G

IgM - Imunoglobulina M

IMC - Índice de massa corpórea

IND - *Investigational New Drug*

MDRO - Multidrug-resistant organisms

MRSA - *Staphylococcus aureus* meticilina resistente

NAAT - Teste de amplificação de ácido nucleico

NASPG - Sociedade Norte-Americana de Gastroenterologia Pediátrica

NASPGHAN - Sociedade Norte-Americana de Gastroenterologia Pediátrica, Hepatologia e Nutrição

OMDR - Organismo multidroga resistente

PCR - Reação em cadeia da polimerase

PEG - Polietilenoglicol

POP - Procedimento operacional padrão

PPI - Proton pump inhibitor

PTTa - Tempo de tromboplastina parcial ativada

RNI - Razão normalizada internacional

SPSS - *Statistical Package for the Social Sciences*

TCLE - Termo de consentimento livre e esclarecido

TGI - Trato gastrointestinal

TMF - Transplante de microbiota fecal

TMO - Transplante de medula óssea

TSH - Hormônio estimulador da tireoide

UFMG - Universidade Federal de Minas Gerais

VDRL - *Venereal Disease Research Laboratory*

VRE - Enterococo resistente à vancomicina

SUMÁRIO

1. TÍTULO

Implantação do Centro de transplante de microbiota fecal do Instituto Alfa de Gastroenterologia do Hospital das Clínicas da UFMG e análise dos primeiros resultados em pacientes com infecção recorrente ou refratária pelo *Clostridioides difficile*

2. RESUMO DO TRABALHO

Introdução: O Transplante de Microbiota Fecal (TMF) é uma importante opção terapêutica para a infecção recorrente ou refratária pelo *Clostridioides difficile*, sendo método seguro e eficaz. Resultados iniciais sugerem que o TMF também desempenha um papel relevante em outras afecções cuja patogênese envolve a alteração da microbiota intestinal. No entanto, seu uso sistematizado é pouco difundido, especialmente no Brasil. Na última década, surgiram múltiplos relatos e séries de casos utilizando diferentes protocolos para o TMF, sem padronização de métodos e com taxas de resposta variáveis. No Brasil, foram relatados poucos casos isolados de TMF, com taxa de sucesso em torno de 90%, realizados de forma experimental, sem a implantação de um Centro de Transplante de Microbiota Fecal (CTMF). É objetivo principal desse estudo descrever o processo envolvido na implantação de um Centro de Transplante de Microbiota Fecal (CTMF) no Instituto Alfa de Gastroenterologia do Hospital das Clínicas da UFMG/EBSERH (IAG-HC/UFMG) para o tratamento de infecção recorrente e refratária pelo *C. difficile* e analisar prospectivamente os resultados do tratamento a curto e longo prazo.

Métodos: O CTMF foi estruturado dentro dos critérios exigidos e aprovados por organismos internacionais como o FDA (*Food and Drug Administration*), Grupo Europeu de Transplante de Microbiota Fecal e em consonância com os aspectos epidemiológicos e regulatórios nacionais.

Resultados: Foi estabelecida plataforma que define todas as etapas envolvidas na seleção de doadores universais, processamento e armazenamento de amostras, uniformização de vias de administração do substrato fecal e seguimento a curto e longo

prazo dos pacientes transplantados. A seleção de doadores foi realizada em três etapas: pré-triagem, avaliação clínica e triagem laboratorial. A maioria dos candidatos foi excluída na primeira (75,4%) e segunda etapa (72,7%). Os principais critérios clínicos de exclusão foram: diarreia aguda recente, excesso de peso (índice de massa corporal ≥ 25 kg / m²) e distúrbios gastrointestinais crônicos. Apenas quatro dos 134 candidatos foram selecionados como doadores após rastreio completo, com taxa de detecção de doadores habilitados de 3%. Ao todo foram realizados 11 transplantes em 10 pacientes com ICD recorrente. A taxa de resolução primária, com apenas um procedimento, foi de 80% e a taxa de remissão geral, após segundo TFM, foi de 90%. A ocorrência de eventos adversos foi semelhante à observada em outros estudos. A maioria dos eventos adversos foram autolimitados e de resolução espontânea.

Conclusão: A implantação de um centro de transplante, inédito no nosso país, permitiu o acesso de pacientes com infecção recorrente pelo *C. difficile* a tratamento inovador, seguro e efetivo. A seleção adequada de doadores qualificados é vital no processo de implantação de um CTMF. A rigorosa avaliação clínica dos doadores permitiu o uso racional de recursos. Um centro de transplante de microbiota possibilita oferecer um tratamento sob demanda, menos personalizado, com mais segurança e rastreabilidade. Mesmo em países emergentes, onde há preocupação com doenças tropicais e infecciosas, o TMF parece ser uma estratégia segura e efetiva no tratamento de ICD recorrente.

Palavras-chave: transplante de microbiota fecal, fezes, *Clostridioides difficile,* infecções por Clostridium

2. ABSTRACT

Introduction: Fecal Microbiota Transplantation (FMT) is an important therapeutic option for recurrent or refractory *Clostridioides difficile* infection, being a safe and effective method. Initial results suggest that FMT also plays a relevant role in other conditions whose pathogenesis involves alteration of the intestinal microbiota. However, its systematic use is not widespread, especially in Brazil. In the last decade, several reports and case series have emerged using different protocols for FMT, without standardization of methods and with variable response rates. In Brazil, few isolated cases of FMT have been reported, with a success rate of around 90%, performed experimentally, without the implementation of a Fecal Microbiota Transplant Center (FMTC). The main objective of this work is to describe the implementation process of a Fecal Microbiota Transplant Center (FMTC) at the Alfa Institute of Gastroenterology, Hospital das Clínicas, UFMG/EBSERH (IAG-HC/UFMG) for the treatment of refractory infection. by C. difficile and prospectively analyze short- and long-term treatment outcomes.

Methods: The FMTC was structured within the criteria required and approved by international organizations such as the FDA (Food and Drug Administration), the European Fecal Microbiota Transplantation Group and in line with national epidemiological and regulatory aspects.

Results: A platform was established that defines all the steps involved in the selection of universal donors, processing and storage of samples, standardization of fecal substrate administration routes and short and long-term follow-up of transplant patients. Donor selection was performed in three stages: pre-screening, clinical evaluation and laboratory screening. Most candidates were excluded in the first (75.4%) and second phases (72.7%). The main clinical exclusion criteria were: recent acute diarrhea, overweight (body mass index $\geq$ 25 kg/m²) and chronic gastrointestinal disorders. Only four of 134 candidates were selected as donors after full screening, with a detection rate of eligible donors of 3%. In all, 11 transplants were performed in 10 patients with recurrent CDI. The primary resolution rate with just one procedure was 80% and the overall remission rate after a second FMT was 90%. The occurrence of adverse events

was similar to that observed in other studies. Most adverse events were self-limiting and resolved spontaneously.

Conclusion: The implementation of a transplant center, unprecedented in our country, provided patients with recurrent C. difficile infection access to an innovative, safe and effective treatment. Proper selection of qualified donors is vital in the process of implementing a CTMF. The rigorous clinical evaluation of the donors allowed the rational use of resources. A microbiota transplant center makes it possible to offer an on-demand, less personalized treatment, with more security and traceability. Even in emerging countries, where there is concern about tropical and infectious diseases, TMF seems to be a safe and effective strategy in the treatment of recurrent CDI.

Keywords: fecal microbiota transplantation, feces, *Clostridium difficile* infection, *Clostridioides difficile*

3. CONSIDERAÇÕES INICIAIS

3.1. MICROBIOTA INTESTINAL HUMANA

A microbiota intestinal é um dos sistemas mais complexos do corpo humano. É composta por 10^{14} micro-organismos e participa de funções importantes à saúde como digestão, imunidade, síntese de vitaminas, fermentação de carboidratos e metabolismo de bile [1,2]. Quando em equilíbrio, desempenha papel na resistência à colonização de patógenos externos. Desenvolve-se a partir do nascimento em um processo dinâmico, influenciado por fatores genéticos e ambientais como via de parto, alimentação, uso de medicamentos, localização geográfica e hábitos de vida. A partir dos três anos de idade a microbiota torna-se mais estável, com menor variabilidade interindividual, permanecendo assim ao longo da idade adulta. Contudo, não mantém sua composição de forma fixa e pode se modificar em resposta a estímulos ambientais e fatores do próprio hospedeiro. É considerada por muitos estudiosos como um órgão metabolicamente ativo, composto por um número de organismos dez vezes maior que o número de células do corpo humano e capaz de exercer sua função no sistema intestinal e extra intestinal [1].

As fezes humanas são compostas por 75% de água e 25% de matéria sólida [3]. Quase metade da porção sólida corresponde a micro-organismos entéricos. Além das bactérias (10^{11} por grama de fezes seca), são encontrados vírus (10^{8} por grama de fezes), arqueias (10^{8} por grama de fezes), colonócitos (10^{7} por grama de fezes), fungos (10^{6} por grama de fezes), protozoários, metabólitos e material genético [3]. Cada componente atua de forma sinérgica para manter a homeostase local entre microbioma e hospedeiro. Até mesmo o DNA bacteriano e células mortas exercem função imunoestimuladora e de equilíbrio ambiental [3].

O conhecimento sobre a microbiota humana tem crescido de forma acelerada e proporcionado descobertas importantes. Com técnicas de sequenciamento genético é possível caracterizá-la com maior acurácia e estudar suas mudanças em situações de adoecimento [2]. O distúrbio de sua homeostase, conhecido como disbiose, tem sido relacionado a patogênese de várias afecções, sendo a infecção pelo *Clostridioides difficile* (ICD) a mais estudada. Nesse sentido, observa-se um esforço progressivo no

desenvolvimento de novas terapias capazes de modular a microbiota, corrigir a disbiose e atuar de forma benéfica no tratamento dessas afecções. Dentre as possibilidades de modulação destaca-se o uso de prebióticos, simbióticos, probióticos e, mais recentemente, o Transplante de Microbiota Fecal (TMF).

3.2. TRANSPLANTE DE MICROBIOTA FECAL

O Transplante de Microbiota Fecal é o procedimento no qual a microbiota hígida, oriunda de doadores saudáveis, é transferida para o trato gastrointestinal de uma pessoa doente a fim de repovoar seu tubo digestivo, corrigir a disbiose subjacente e participar do processo de recuperação do doente. Ao contrário dos probióticos, o material introduzido é composto por toda diversidade de espécies e metabólitos presentes nas fezes do doador, capaz de exercer suas funções por prazo prolongado [4]. O TMF pode ser realizado por meio de diversos métodos como comprimidos orais, sondas nasogástricas, nasoentéricas, via endoscópica, colonoscópica ou por enemas. Não há, até o momento, nenhum estudo que indique a superioridade de um método sobre outro [5].

3.2.1. HISTÓRIA DO TRANSPLANTE DE MICROBIOTA FECAL

Apesar do entusiasmo na medicina moderna, a utilização de fezes saudáveis para tratamento de pessoas doentes tem sido descrita desde a antiguidade [6]. Os primeiros registros sobre transplante fecal remontam o século IV, na China. Durante a dinastia de Dong Jin, o médico Ge Hong descreveu pela primeira vez, em seu manual de medicina "*Zhou Hou Bei Ji Fang*", a ingestão de suspensão fecal humana para tratamento de intoxicação alimentar e diarreia grave. Posteriormente, no século XVI, o médico Li Shizhen documentou em seu livro de medicina tradicional "*Ben Cao Gang Mu*" a utilização de fezes secas, suspensões fecais fermentadas e até fezes frescas de crianças para tratamento de diarreia grave, febre, vômitos e constipação intestinal. Para melhor aceitação, esse tratamento recebia o nome de "sopa amarela" ou "xarope dourado" [6].

Durante a segunda guerra mundial, os soldados alemães na África (Afrika Korps) foram orientados a utilizar fezes de camelo para tratamento de disenteria bacteriana. Enquanto muitos soldados morriam com disenteria, a população local se protegia consumindo

fezes frescas e quentes de camelos ao primeiro sinal de doença. A partir dessa observação, cientistas nazistas analisaram as fezes e conseguiram isolar o *Bacillus subtilis*, utilizado posteriormente pela corporação com bons resultados [7].

A primeira descrição de TMF na medicina moderna remonta a 1958. Ben Eiseman usou de forma bem sucedida enemas fecais para tratamento de quatro pacientes com colite pseudomembranosa grave refratária ao uso de antibióticos [8]. Mesmo sem confirmação microbiológica, é provável que os doentes apresentassem infecção pelo *C. difficile.*

Em 2013 foi publicado o primeiro ensaio clínico controlado e randomizado utilizando TMF para pacientes com ICD recorrente [9]. Pacientes com infecção recorrente pelo *C. difficile* foram randomizados para receber uma das três terapias: (1) vancomicina oral 500mg de 6/6h por quatro dias seguido de lavagem intestinal e subsequente TMF por sonda nasoentérica; (2) terapia com vancomicina oral isolada (500mg 6/6h via oral por 14 dias) ou (3) terapia com vancomicina oral acrescida de lavagem intestinal. O estudo teve que ser interrompido após a análise inicial dos dados, frente à elevada eficácia do TMF. Dos 16 pacientes do grupo do TMF, 13 (81%) apresentaram resolução da diarreia após a primeira infusão. A resolução da diarreia no grupo de vancomicina isolada e vancomicina com lavagem intestinal foi de 31 e 23% respectivamente ($p < 0,001$ comparados ao grupo do TMF). O TMF foi significativamente mais efetivo que o tratamento padrão. A partir desse ensaio clínico randomizado a eficácia do TMF foi comprovada por outros estudos. Atualmente, o TMF se tornou terapia padrão para ICD recorrente e encontra-se em investigação para tratamento de outras doenças.

3.3. INDICAÇÕES DE TRANSPLANTE DE MICROBIOTA FECAL

O TMF tem se estabelecido como terapia promissora para casos ICD e tem mudado conceitos sobre o manejo desses pacientes, especificamente na prevenção de novas recorrências. Com as informações obtidas a partir de série de casos, ensaios clínicos randomizados e meta-análises, o Consenso Europeu sobre Transplante de Microbiota Fecal e a Sociedade Americana de Doenças Infecciosas passaram a recomendar o TMF como tratamento para ICD refratária ou recorrente, especialmente a partir da segunda recorrência [10,11]. Não há dados suficientes para recomendá-lo como tratamento para o primeiro episódio, nem ao menos como adjuvante à antibioticoterapia [12]. Marie

Hocquart *et al*. propõem que o TMF seja considerado como tratamento de primeira linha para casos graves de ICD, com base na significativa redução de mortalidade em três meses [13]. No entanto, estudos adicionais são necessários para tornar essa proposta consensual.

São várias as afecções cujo transplante tem sido testado como opção terapêutica, ainda que em caráter experimental e, entre elas, destaca-se seu emprego em doença inflamatória intestinal, síndrome do intestino irritável, obesidade, resistência periférica à insulina, afecções hepatobiliares, hemato-oncológicas, infecções por organismos multidroga resistentes e síndromes neurológicas [14]. No entanto, a infecção recorrente ou refratária pelo *C. difficile* representa a principal indicação ao transplante, haja vista o volume e força das evidências [11]. O tratamento tem eficácia em torno de 90% na eliminação de ICD recorrente e está associado a poucos efeitos colaterais, em sua grande maioria, leves e transitórios [9,15-18]. Apresenta melhora da qualidade de vida e é bem aceito pelos pacientes [9,15-19]. Contudo, uma minoria dos casos não responde satisfatoriamente. Fatores relacionados aos doadores, receptores e ao próprio procedimento em si podem contribuir para o insucesso do tratamento e demandam investigação. Elementos que podem influenciar negativamente o resultado do transplante são: baixo volume de fezes, colite grave, colite com evidência endoscópica de pseudomembranas, uso concomitante de outros antibióticos e hospitalização [11,12].

Para suprir a demanda de tratamento, é necessária a implantação de centros de transplante e bancos de fezes capacitados a fornecer material e executar o procedimento de forma ágil e segura. Atento à crescente implantação de Centros de Transplante Fecal, o *Food and Drug Administration* (FDA), em 2013, regulamentou o TMF como nova modalidade de tratamento e estabeleceu recomendações para sua aplicação [20]. No mesmo ano, a Sociedade Americana de Doenças Infecciosas (IDSA), Sociedade Americana de Endoscopia Gastrointestinal (ASGE), Sociedade Norte-Americana de Gastroenterologia Pediátrica (NASPG), Hepatologia e Nutrição (NASPGHAN), Associação Americana da Gastroenterologia (AGA) e Colégio Americano de Gastroenterologia (ACG) emitiram orientações consensuais para regulamentar o rastreio e análise das fezes de doadores [21]. Entretanto, apesar do esforço quanto a padronização, surgiram vários centros de transplante e bancos de fezes independentes, com protocolos heterogêneos. Preocupado com a falta de controle e uniformização acerca do

procedimento, o FDA emitiu em 2016 orientações específicas sobre segurança em bancos de fezes [19].

A recomendação atual é que o TMF seja executado em centros de referência para tratamento de ICD, especialmente em Hospitais com experiência no tratamento de *C. difficile* e logística apropriada [12,22]. O centro deve ser composto por equipe multidisciplinar capitaneado por médico gastroenterologista, microbiologista ou infectologista, com conhecimento científico apropriado e experiência com TMF. O diretor do banco deve garantir que o fornecimento de amostras fecais na prática clínica seja apenas para tratamento de ICD e a participação em protocolos de pesquisa para outras indicações é aceito somente após aprovação do projeto em rigorosa análise pelo comitê de ética local [22]. O banco de fezes deve contar ainda com a participação de especialista em biobancos capaz de processar e armazenar as amostras em condições padronizadas e garantir o cumprimento de padrões de qualidade exigidos no processo [22].

3.3.1. INFECÇÃO RECORRENTE OU REFRATÁRIA PELO *C. DIFFICILE*

O *Clostridium difficile* teve sua nomenclatura recentemente modificada e passou a ser denominado *Clostridioides difficile.* Representa o principal patógeno responsável por diarreia associada aos cuidados à saúde humana [23]. É um bacilo Gram-positivo, formador de esporos, cujas toxinas causam doença gastrointestinal com amplo espectro de gravidade. O quadro clínico varia desde diarreia leve, colite pseudomembranosa até megacólon tóxico, podendo levar a óbito. Sua incidência, gravidade e recorrência têm aumentado em todo o mundo ao longo das últimas décadas [24]. O uso indiscriminado de antibióticos, especialmente as quinolonas, a maior longevidade da população e o surgimento de estirpes hipervirulentas, responsáveis por grandes epidemias ao redor do mundo, tem contribuído para o aumento da morbimortalidade associada a infecção pelo *C. difficile* [25]. A taxa de mortalidade nos Estados Unidos aumentou de 1,5% para 6%, atingindo 17% em períodos de epidemia [24]. Nos casos de ICD grave, a mortalidade é em torno de 36-58% [26,27]. Habitualmente, a infecção é tratada com antibióticos como metronidazol, vancomicina ou fidaxomicina, com taxa de recidiva em torno de 25-30% [28]. No entanto, pacientes que apresentam recidivas subsequentes, possuem uma chance

de desenvolver uma nova recorrência de até 60%, mesmo quando adequadamente tratados com antibióticos [28]. A base fisiopatológica para esse comportamento não se restringe à existência de resistência antimicrobiana, mas sobremaneira à incapacidade de reestabelecer uma microbiota intestinal saudável que impeça o desenvolvimento da nova infecção [29].

O mecanismo envolvido na recorrência da infecção pelo *C. difficile* consiste na reexposição ou reativação dos esporos em pacientes com disbiose, resposta imune deficitária e defeito na função de barreia do epitélio colônico [30]. Os pacientes com ICD recorrente possuem uma microbiota alterada em sua composição e reduzida em sua diversidade, geralmente resultado de exposição a antibióticos usados previamente para tratamento de outra afecção ou até mesmo no tratamento da colite pelo *C. difficile* [31]. A antibioticoterapia tradicional utilizada na ICD pode gerar um ciclo de disbiose ao perpetuar o desequilíbrio da microbiota favorecendo um ambiente propício a proliferação do *C. difficile*. O exato mecanismo de ação do TMF ainda não foi totalmente elucidado, mas o racional do transplante é que ele seja capaz de quebrar o ciclo de disbiose ao introduzir uma nova microbiota, saudável e rica em diversidade, apta a ocupar o nicho intestinal e impedir o desenvolvimento do *C. difficile*. No entanto, a microbiota não é o único determinante para o sucesso terapêutico do transplante. Estudo conduzido por Ott. *et al.* em 2017 mostrou que o transplante com substrato fecal filtrado e estéril, livre de bactérias viáveis, também é eficaz no tratamento contra ICD recorrente [32]. Tal achado sugere que substâncias não bacterianas como proteínas, compostos antimicrobianos, produtos metabólicos e oligonucleotídeos também contribuam para o efeito terapêutico do TMF.

3.4. CENÁRIO NACIONAL SOBRE INFECÇÃO PELO *C. DIFFICILE* E TRANSPLANTE DE MICROBIOTA FECAL

No Brasil, a ICD é reconhecida como a principal causa de diarreia nosocomial relacionada ao uso de antibióticos [33]. Apesar da subnotificação e dos poucos dados epidemiológicos nacionais, é crescente o número de registros sobre isolamento e caracterização do *C. difficile* no nosso meio. Cançado *et al.,* em 2018, avaliaram coorte de adultos internados em hospital universitário de Belo Horizonte que desenvolveu

diarreia após uso de antibióticos. A prevalência de ICD foi de 31,8% e esteve relacionada a comorbidades subjacentes e ao número de antibióticos utilizados duração a hospitalização. A quase totalidade das cepas toxigênicas apresentaram os genes tcdA e tcdB. Os principais ribotipos de PCR identificados foram 014/020 e 106. Foram encontradas estirpes produtoras de toxina binária não associadas aos ribotipos 027 e 078 [34].

No mesmo ano, no Brasil, foi isolada pela primeira vez a estirpe hipervirulenta do *C. difficile* ribotipo 027 (NAP1/027) [35]. A cepa foi responsável pelo aumento dos casos mundiais de ICD a partir de 2000, com surtos na América do Norte e Europa [33]. O ribotipo epidêmico já havia sido isolado na Austrália, Ásia, América Central e América do Sul, mas ainda não no Brasil. No entanto, apesar de não haverem relatos de surtos nacionais até o momento, a identificação do novo ribotipo hipervirulento 027 e outras estirpes produtoras de toxina binária no país lança o alerta para necessidade de otimização de medidas preventivas, difusão de métodos diagnósticos e facilitação para acesso a medidas terapêuticas, em especial, ao TMF.

Apesar do advento de casos de infecções recorrentes de *C. difficile* no Brasil, o TMF ainda não é uma realidade na prática clínica nacional. São poucos os relatos de transplante fecal no nosso país. Até o momento, apenas um estudo foi publicado, no ano de 2015, descrevendo a experiência de 12 pacientes com ICD submetidos ao transplante em São Paulo, com taxa de sucesso de 90% [36]. Além disso, há uma escassez de dados sobre triagem de doadores em países emergentes, especialmente no Brasil.

3.5. ASPECTOS REGULATÓRIOS SOBRE TRANSPLANTE DE MICROBIOTA FECAL E BANCO DE FEZES

Nos Estados Unidos, em maio de 2013, o FDA regulamentou o TMF como um novo medicamento sob investigação (*U.S. Investigational New Drug*, IND) [20]. Tal medida foi recebida pela comunidade médica e pelos pacientes com certa preocupação uma vez que limitava o acesso a modalidade promissora de tratamento. Mais tarde, em julho de 2013, a agência alterou sua declaração, liberando os pacientes com ICD recorrente da necessidade de aplicação do IND, desde que fosse utilizado Termo de Consentimento Livre e Esclarecido (TCLE) [21]. O termo aborda riscos, benefícios, alternativas ao

tratamento e deve explicitar que o transplante fecal é um tratamento sob investigação. O doador e as fezes devem passar por testes de triagem realizado pelo prestador do serviço adequadamente capacitado [20]. A liberação não incluiu as outras indicações de transplante. Para realizar o TMF para outras afecções, é necessário que as instituições enviem uma solicitação de IND.

Na Europa, em dezembro de 2014, a Comissão Europeia considerou as fezes utilizadas no transplante como um "produto combinado", composto por células humanas e componentes não humanos, como o microbioma [37]. No entanto, considerando que o componente humano não é o principal responsável pela resposta terapêutica do TMF, a Comissão decidiu que o substrato fecal não se enquadra nas diretrizes da *European Tissue and Cells Directive*. Por conseguinte, as autoridades competentes permitiram que o regulamento fosse gerido a nível nacional de execução. Os estados membros são livres para criar estruturas regulatórias específicas para o transplante de células e tecidos em seus territórios e cada banco de fezes deve operar sob as regulações de cada país.

No Brasil, assim como em vários países do mundo, não há regulamentação específica para o TMF. O Consenso Internacional sobre Transplante de Microbiota Fecal recomenda que, na ausência de diretrizes locais, o transplante seja realizado sob a égide de um banco de fezes com comitê científico responsável [22]. O banco deve contar com médico para avaliar, selecionar e recrutar doadores de fezes; microbiologista e/ou farmacêutico para coordenar todos os procedimentos relacionados ao processamento de fezes e armazenamento; um especialista em biobancos para armazenar adequadamente as amostras fecais e um diretor para garantir o cumprimento de todas as etapas. Por ser um tratamento sob investigação, recomenda-se que o TMF seja realizado nos moldes de estudo científico. De acordo com a legislação que rege estudos envolvendo seres humanos, é necessária a aprovação prévia do protocolo de pesquisa pelo Comitê de Ética em Pesquisa (CEP) da instituição.

4. OBJETIVOS

4.1. OBJETIVO GERAL

Implantar o Centro de Transplante de Microbiota Fecal do IAG-HC/UFMG visando o tratamento de pacientes com infecção recorrente ou refratária pelo *C. difficile*, capacitado para casos regionais e de todo país.

4.2. OBJETIVOS ESPECÍFICOS

a) Descrever o processo de estruturação do Centro de Transplante de Microbiota Fecal com banco de fezes congeladas.

b) Definir os critérios a partir de um protocolo para seleção dos doadores.

c) Estabelecer o procedimento de preparo e armazenamento das amostras fecais.

d) Determinar procedimentos para administração do substrato fecal.

e) Realizar os primeiros transplantes de microbiota fecal.

f) Criar uma plataforma para estudos futuros no campo da microbiota intestinal.

g) Analisar os resultados clínico-laboratoriais dos pacientes submetidos ao TMF.

5. MÉTODOS

5.1. INFRAESTRUTURA DO CENTRO DE TRANSPLANTE DE MICROBIOTA FECAL

O centro de transplante de microbiota fecal IAG-HC/UFMG foi estruturado no âmbito do banco de tumores e tecidos da instituição, desenvolvido dentro de critérios exigidos e aprovados por organismos internacionais como o FDA, Grupo Europeu de Transplante de Microbiota Fecal e de acordo com os aspectos regulatórios nacionais. Neste processo, contou com o apoio do Laboratório de Bacteriose do Departamento de Medicina Veterinária Preventiva da UFMG e do setor de endoscopia do IAG-HC/UFMG. O hospital das clínicas da UFMG é um hospital universitário, público federal, gerido pela Empresa Brasileira de Serviços Hospitalares (EBSERH) e participa de atividades relacionadas ao ensino, pesquisa e assistência. O CTMF é formado por equipe multidisciplinar com gastroenterologistas experientes em ICD, especialista em microbiologia e *C. difficile,* farmacêutica e biomédica com vivência em biobanco, gastroenterologista e endoscopista responsável pela avaliação dos doadores, receptores e realização dos transplantes.

O banco de tumores e tecidos do IAG-HC/UFMG, é um biobanco encarregado de reunir, de forma organizada, material biológico humano, coletado e armazenado para fins de pesquisa, sob responsabilidade e gerenciamento institucional, sem fins comerciais, conforme as diretrizes e regulamentações nacionais presentes na Resolução CNS n°466/12, Resolução CNS n°441/11 e Portaria 2.201/2011 do Ministério da Saúde e complementares. Foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais em 11/08/2010 (Parecer n°. ETIC 0163.203.000-10) e também pela Comissão Nacional de Ética em Pesquisa (CONEP) em 13/06/2019, registro CONEP B-069, processo nº 25000.185396/2016-52.

5.2. DESENHO DO ESTUDO

O presente trabalho foi aprovado pelo CEP-UFMG (CAAE 72755217.8.0000.5149 – parecer 2.264.667 em 08/09/2017) (Anexo A), sendo dividido em duas etapas. Primeiramente, foi realizada revisão da literatura sobre aspectos relevantes na

estruturação de um centro de transplante de microbiota fecal com banco de fezes congeladas. Foram pesquisados artigos acoplados aos bancos eletrônicos *PubMed*, *Lilacs*, *MEDLINE* e *Cochrane* e selecionados conforme título, resumo e relevância no campo do TMF. Os principais descritores foram *Clostridium difficile*, recurrent *Clostridium difficile* infection, fecal microbiota transplantation, fecal transplantation, intestinal microbiota transplantation, donor selection, frozen stool e stool bank. A busca foi limitada a estudos publicados em inglês e português até setembro de 2017. Paralelamente, foram realizadas reuniões entre os membros da equipe do CTMF para delineamento de protocolos, realização de Procedimento Operacional Padrão (POP), revisão e análise das medidas já implantadas e definição de novas diretrizes. Foram selecionados doadores e executada metodologia para preparo e armazenamento do substrato fecal. Após aquisição das primeiras amostras, o funcionamento do CTMF foi divulgado em meios de comunicação de alcance estadual e nacional como telejornais, rádios, revistas e redes sociais da Federal Brasileira de Gastroenterologia e Associação Mineira de Gastroenterologia. Foram realizadas palestras e afixados cartazes em hospitais de Belo Horizonte para divulgação no município.

A segunda etapa do trabalho consistiu na condução de um estudo piloto prospectivo, aberto, não controlado, em centro único, para avaliar a eficácia do transplante de microbiota fecal em pacientes com ICD recorrente ou refratária. Os pacientes receberam o transplante de doadores saudáveis selecionados na primeira etapa. Foram avaliadas variáveis clínicas, demográficas, comorbidades, exposição prévia a medicamentos, gravidade da ICD e dados laboratoriais de dez pacientes antes e após o tratamento. Buscou-se relatar a experiência inicial com os transplantes e determinar taxa de resolução e segurança a curto e longo prazo.

5.3. SELEÇÃO DOS DOADORES

A seleção dos doadores foi realizada prospectivamente em uma abordagem de três etapas: (1ª) recrutamento e triagem; (2ª) avaliação clínica dos possíveis doadores e (3ª) avaliação laboratorial com exames de sangue e fezes. Foram utilizados como base para seleção de doadores os critérios estabelecidos pelo FMT Working Group [38], protocolo de Amsterdam [9], protocolo australiano [39], as orientações consensuais sobre triagem na

carta das sociedades americanas ao FDA [40], Consenso Europeu sobre TMF na prática clínica [12], Consenso Internacional sobre Banco de Fezes para TMF [22] e critérios adotados pelo OpenBiome [41]. Os critérios de seleção foram realizados de acordo com as recomendações consensuais entre os protocolos acrescidos de especificidades epidemiológicas brasileiras.

5.3.1. RECRUTAMENTO E TRIAGEM

Foi realizada triagem de voluntários para avaliação de elegibilidade como doadores de fezes. Os candidatos receberam o convite para participação voluntária e foram submetidos a uma autoavaliação que abordou quatro questões: (1) presença de alguma doença conhecida; (2) problemas com o peso corporal; (3) queixas digestivas recorrentes e (4) indisponibilidade logística para a doação de fezes. A presença de pelo menos um desses critérios inviabilizou a continuidade no processo de seleção de doadores. Os aprovados prosseguiram para a segunda etapa na condição de potenciais doadores.

5.3.2. AVALIAÇÃO CLÍNICA DOS POTENCIAIS DOADORES

A avaliação clínica foi realizada por único pesquisador e consistiu na entrevista médica completa com detalhamento sobre histórico de saúde, exame físico e análise de critérios de inclusão e exclusão. Os candidatos foram submetidos a questionário semelhante ao utilizado em doação de sangue (Apêndice A). Apenas os doadores aprovados nessa etapa foram submetidos à realização de exames de sangue e fezes.

5.3.3. AVALIAÇÃO LABORATORIAL DOS POTENCIAIS DOADORES

5.3.3.1. EXAMES DE SANGUE

Os potenciais doadores foram submetidos aos seguintes exames de sangue: hemograma completo, proteína C reativa, ureia, creatinina, sódio, potássio, cloro, magnésio, cálcio, glicose, aspartato aminotransferase (AST), alanino aminotransferase (ALT), gama glutamil transpeptidase (GGT), bilirrubinas, fosfatase alcalina, albumina, atividade de protrombina com razão normalizada internacional (RNI), tempo de tromboplastina

parcial ativada (PTTa), colesterol total e frações, triglicérides, hormônio estimulador da tireoide (TSH), T4 livre, 25-hidroxivitamina D, ácido fólico, vitamina B12. Exames sorológicos para sífilis (*Veneral Disease Research Laboratory* - VDRL), hepatites A, B e C (pesquisa de anticorpos), vírus da imunodeficiência humana - HIV 1 e 2 (ensaio combinado de anticorpos e antígeno), vírus T-linfotrópico humano - HTLV 1 e 2 (pesquisa de anticorpos), doença de Chagas (pesquisa de anticorpos com dois métodos combinados: hemaglutinação e imunofluorescência indireta) e esquistossomose (pesquisa de anticorpos).

5.3.3.2. EXAMES DE FEZES

Os potenciais doadores foram submetidos aos seguintes exames de fezes: pesquisa do *C. difficile* (glutamato desidrogenase - GDH e cultura toxigência), Norovírus (PCR), Rotavírus (PCR), Coronavírus (PCR), cultura de patógenos entéricos (*Salmonella* sp.*, Shigella* sp.*, Campylobacter* sp.*, Vibrio cholerae, Yersinia* sp.*),* pesquisa de *Escherichia coli* O157 produtora de toxina shiga (isolamento e PCR), *Salmonella* sp. (isolamento e PCR), *Clostridium perfringens* (isolamento e PCR), *Campylobacter* sp. (PCR), cultura para *Staphylococcus aureus* meticilina resistente (MRSA), enterococo resistente a vancomicina (VRE), enterobactérias produtoras de betalactamase de espectro expandido (ESBL), enterobactérias produtoras de carbapenemase, microscopia para ovos e parasitas em três amostras seriadas, pesquisa de *Giardia lamblia* (microscopia e pesquisa de antígeno), *Strongyloides stercoralis* (microscopia e Baermann-Moraes), *Entamoeba histolytica* (microscopia e pesquisa de antígeno), *Schistossoma mansoni* (microscopia), *Cryptosporidium* sp. (microscopia), Isospora (microscopia) e Microscorídeos (microscopia).

5.3.4. CRITÉRIOS DE INCLUSÃO DO DOADOR

Indivíduos adultos, de ambos os sexos, aparentados ou não com o receptor, com idade entre 18 e 50 anos e que concordaram com processo de seleção e doação de fezes mediante assinatura do Termo de Consentimento Livre e Esclarecido – TCLE (Apêndice B).

5.3.5. CRITÉRIOS DE EXCLUSÃO DO DOADOR

As doenças ou condições que excluíram de forma permanente ou transitória um potencial doador foram:

- infecção ativa não controlada durante a doação;
- febre de origem desconhecida ou febre nas últimas duas semanas;
- exposição a antibióticos, imunossupressores ou quimioterápicos nos últimos três meses;
- doença transmissível ativa (HIV, hepatite B, hepatite A ou hepatite C);
- exposição conhecida ou história prévia de HIV 1 e 2, hepatite B, hepatite C, sífilis, HTLV 1 e 2, malária, doença de Chagas, tuberculose, herpes muco cutânea;
- histórico de queixas ou doenças gastrointestinais, incluindo doença inflamatória intestinal, síndrome do intestino irritável, doença celíaca, diarreia ou constipação intestinal crônica, neoplasias malignas gastrointestinais, síndromes polipoides, excesso de gases, flatulência ou grandes procedimentos cirúrgicos gastrointestinais;
- história prévia de transplante de órgãos e tecidos (incluindo córnea);
- história de transfusão sanguínea nos últimos seis meses;
- história de acidente perfuro-cortante nos últimos seis meses;
- história recente (últimos dois meses) de vacinação com vírus vivo atenuado;
- histórico de doenças autoimunes, atópicas ou terapia imunomoduladora em curso;
- fatores de risco para Doença de Creutzfeldt-Jacob (história pessoal prévia ou familiar, receptores de enxerto como transplante de córnea, uso prévio de hormônios de pituitária cadavérica, uso prévio de insulina bovina, exposição nosocomial, pessoas que permaneceram no Reino Unido e/ou Irlanda por mais de três meses entre 1980 e 1996 ou que tenham permanecido por mais de cinco anos, consecutivos ou intermitentes, na Europa após 1980 até os dias atuais);
- profissionais da área de saúde expostos ao risco de transmissão de doenças infecciosas ou risco de serem carreadores de organismos multidroga resistentes (OMDR);
- profissionais que trabalham com animais, sob risco de transmissão de zoonoses;

- comportamento sexual de alto risco (contato sexual com anônimos, contato sexual com profissionais do sexo, uso de drogas antes da relação sexual, contato sexual com indivíduos com HIV ou hepatites virais, homem que faz sexo com homem, relação com homem bissexual, múltiplos parceiros sexuais e profissionais do sexo);
- novo contato sexual nos últimos 12 meses;
- história prévia de doença sexualmente transmissível;
- uso de drogas ilícitas endovenosas ou inalatórias;
- história recente de hospitalização (por mais de dois dias nos últimos três meses), encarceramento ou permanência em casas de repouso;
- implante de *piercing,* brincos, realização de tatuagens ou acupuntura nos últimos seis meses;
- história recente de hematoquezia ou outros sangramentos do trato gastrointestinal (últimos dois meses);
- doença diarreica aguda recente nos últimos seis meses;
- sobrepeso e obesidade definidos pela Organização Mundial de Saúde como Índice de Massa Corpórea (IMC) maior ou igual a 25 e 30 kg/m² respectivamente;
- desnutrição moderada a grave;
- diabetes mellitus;
- síndrome metabólica definida como a presença de pelos menos três dos seguintes critérios: (1) circunferência abdominal acima de 102 cm em homens e 88 cm em mulheres; (2) níveis de *High Density Lipoprotein* (HDL) abaixo de 40 mg/dL em homens e 50 mg/dL em mulheres; (3) níveis de triglicerídeos acima de 150 mg/dL; (4) níveis de glicemia de jejum acima de 110 mg/dL; (5) pressão arterial maior que 130/85 mmHg ou se está em vigência de medicamento anti-hipertensivo);
- transtornos psiquiátricos;
- síndromes de dor crônica (fibromialgia, fadiga crônica) ou síndromes neurológicas;
- histórico de neoplasias malignas;
- uso crônico de inibidores de bomba de prótons (IBP) por no mínimo três meses;

- história familiar de síndrome polipoide ou câncer colorretal prematuro (abaixo de 50 anos) em parente de primeiro grau;
- detecção de alguma alteração nos exames de sangue;
- detecção de algum patógeno nos exames de fezes.

5.4. COLETA DO SUBSTRATO FECAL

Foi recomendado aos doadores que fizessem coletas semanais no primeiro mês e a cada 15 dias nos três meses seguintes. No momento da coleta de fezes, os doadores foram avaliados sobre suas condições de saúde desde o último rastreio. A cada doação foi realizado contato telefônico com abordagem dos seguintes fatores de risco: (1) desenvolvimento de diarreia; (2) presença de alguma doença ou queixa; (3) uso de antibióticos ou novos medicamentos; (4) novo contato sexual. Doadores com sintomas de infecção ativa ou com um dos fatores de risco citados acima, foram excluídos de forma temporária. Passado o período definido nos critérios de exclusão (seis meses para diarreia, três meses para antibióticos, 12 meses para novo contato sexual), o candidato foi convocado e, caso concordasse, submetido a novo processo de rastreio.

As coletas foram realizadas durante quatro meses após aprovação no processo de seleção de doadores. Após esse período, o doador era convidado a permanecer no programa. Para isso era necessário que fosse submetido a novo rastreio com avaliação clínica completa e realização de exames de sangue e fezes.

As fezes doadas foram coletadas em frascos de exame de rotina (50 mL / 50 g de fezes) conforme procedimento padrão de coleta de fezes, em superfície plástica limpa e seca e no ambiente domiciliar. O material identificado com nome do doador, data e horário da coleta foi enviado ao laboratório do CTMF dentro do prazo máximo de duas horas. O profissional responsável pelo recebimento e preparo do material utilizou avental, luvas, máscara e proteção facial durante o manuseio.

5.5. PROCESSAMENTO E ARMAZENAMENTO DAS AMOSTRAS

O procedimento de preparo das fezes para o TMF foi conduzido em espaço adequado e exclusivo (nível 2 de risco biológico). Após pesagem, as fezes foram transferidas para

recipiente com tampa contando solução salina não bacteriostática (sem conservantes) a 0,9% na proporção de 50 g de fezes para cada 250 mL de solução salina. A mistura foi homogeneizada manualmente durante dois a cinco minutos. A suspensão foi transferida cuidadosamente para outro recipiente preparado previamente com filtro de gaze composto por funil, cinco gazes abertas sobrepostas e elástico para fixação. A suspensão foi filtrada em gaze por duas vezes, com o objetivo de retirar fibras alimentares e sujidades grosseiras que poderiam obstruir o canal de trabalho do colonoscópio. Após filtração, adicionou-se glicerol com concentração final de 10% para crioproteção (prevenção de formação de cristais). As suspensões fecais foram então acondicionadas em recipientes plásticos com tampa e armazenadas em ultra-freezer a temperatura de –80°C até o uso. O tempo de viabilidade estabelecido desde o preparo até a administração foi de seis meses.

5.6. DESCONGELAMENTO E PREPARO DO MATERIAL PARA INFUSÃO

No dia da realização do transplante, 250 a 300 mL de suspensão fecal foi retirada do ultra-freezer e descongelada. As alíquotas foram descongeladas à temperatura ambiente, a 4ºC e/ou em banho-maria a 37 °C. O método escolhido para descongelamento variou conforme o horário do procedimento. Após o descongelamento completo, o material foi transferido para seringas de 60 mL, sem agulha, com auxílio de sonda de aspiração calibre 14 French. As seringas foram vedadas, identificadas e acondicionadas em recipiente próprio (cuba em inox) para transporte dentro de caixa de isopor contendo gelo em gel. Uma vez descongelada, a suspensão fecal deveria ser utilizada em até seis horas se à temperatura ambiente ou até oito horas sob refrigeração. As amostras não poderiam ser novamente congeladas caso não fossem utilizadas.

5.7. CRITÉRIOS DE INCLUSÃO DO RECEPTOR

Foram incluídos pacientes com infecção recorrente ou refratária pelo *C. difficile* e que concordaram em participar da pesquisa mediante preenchimento do TCLE (Apêndice C).

5.7.1. DEFINIÇÃO DE INFECÇÃO RECORRENTE E REFRATÁRIA PELO *C. DIFFICILE*

Infecção recorrente foi definida como o desenvolvimento de nova infecção pelo *C. difficile* dentro de oito semanas a partir de um episódio prévio adequadamente tratado, em que houve resolução inicial dos sintomas. A recorrência foi caracterizada pela presença de diarreia, com mais de três dejeções diárias, com fezes não formadas (Bristol 6 ou 7), em um período mínimo de 48h, e resultado laboratorial positivo por meio de GDH ECO Teste – TR.0032 (Eco Diagnóstica, Minas Gerais, Brasil) confirmado por cultura toxigênica [42]. Infecção refratária foi definida como infecção persistente, sem melhora dos sintomas, a despeito do tratamento antimicrobiano com vancomicina oral por no mínimo cinco dias.

5.7.2. DEFINIÇÃO DE GRAVIDADE DA INFECÇÃO PELO *C. DIFFICILE*

- ICD complicada: infecção complicada com megacólon tóxico, peritonite, instabilidade hemodinâmica, insuficiência respiratória ou necessidade de tratamento cirúrgico.
- ICD grave: presença de um dos seguintes critérios (diarreia sanguinolenta, colite pseudomembranosa, íleo adinâmico, dor abdominal intensa, febre com temperatura axilar superior a 38,9 °C, albumina sérica abaixo de 2,5 g/dL, contagem global de leucócitos superior a 20.000 células/mm³, insuficiência renal aguda definida por elevação da creatinina sérica > 0,3 mg/dL em período de 48h).
- ICD leve a moderada: diarreia sem critérios adicionais que caracterizem quadro grave ou complicado [43].

5.8. CRITÉRIOS DE EXCLUSÃO DO RECEPTOR

- Gravidez.
- Choque séptico definido como: sepse com necessidade de vasopressor para elevar a pressão arterial média ≥ 65 mmHg e lactato > 2 mmol/L a despeito de expansão volêmica adequada.
- Expectativa de vida menor que três meses;

- Pacientes incapazes de se submeterem à colonoscopia.
- Incapacidade de preenchimento do TCLE (próprio paciente ou familiar responsável).
- Ausência de critérios para diarreia recorrente ou refratária pelo *C. difficile*.
- Aqueles que recusarem ou desistirem de participar da pesquisa.

5.9. CÁLCULO AMOSTRAL PARA RECEPTORES

Baseado no trabalho de Dias *et al.*, que encontrou taxa de internação por infecção pelo *C. difficile* de 3,3 para 1000 admissões no ano de 2010, número de 18840 internações/ano no HC-UFMG, frequência de infecção anual baseada em estatística americana de 0,0026%, limite de confiança de 5% e o efeito de desenho de 1.0 para amostra aleatória, tem-se um tamanho amostral igual a um paciente [44-46]. Frente a uma doença cuja incidência é baixa, o cálculo amostral não permite avaliar de maneira adequada a estatística inferencial do seu dado. Diante disso, a amostra do estudo foi intencional em cinco receptores e qualquer resultado foi valorizado como experiência inicial.

5.10. MANEJO DOS RECEPTORES ANTES DO TMF

Os candidatos ao transplante passaram por entrevista médica para caracterização da história clínica. As seguintes variáveis foram avaliadas: comorbidades pré-existentes, escore de Charlson, medicamentos em uso, histórico de alergias, duração dos sintomas, número de evacuações ao dia, formato das fezes de acordo com a escala de Bristol e tratamentos prévios para ICD [47,48]. Uma amostra de fezes foi coletada para confirmação diagnóstica e armazenamento. O diagnóstico foi confirmado por meio do GDH e cultura toxigêncica. Os pacientes receberam vancomicina oral 125 mg, de 6/6h, por 10 a 14 dias antes do TMF a fim de reduzir a população intestinal de *C. difficile*. A vancomicina foi interrompida com intervalo de 12 a 24h antes do procedimento. Paciente e familiares foram orientados quanto à desinfecção dos banheiros domiciliares com agente esporicida. Recomendou-se utilização de hipoclorito de sódio a 0,525% (solução de água com água sanitária na proporção 9:1) para limpeza do banheiro,

maçanetas, vaso sanitário, pias e torneiras no dia do procedimento. O paciente foi orientado a não utilizar o banheiro até que fosse realizado o TMF.

5.11. PREPARO INTESTINAL E VIA DE ADMINISTRAÇÃO

Os receptores receberam preparo intestinal habitual para colonoscopia com polietilenoglicol (PEG 4000), dimeticona e bisacodil. Foi utilizado laxativo dividido em duas doses: 120 g de PEG diluídos em um litro de água e administrados via oral na noite da véspera e na manhã do procedimento. Um frasco de 15 mL de dimeticona a 75 mg/mL foi diluído na segunda dose do preparo. Foram utilizados ainda quatro comprimidos de bisacodil 5 mg e dieta sem resíduo na véspera. O jejum recomendado foi de 8h para pequenas refeições e 2h para líquidos claros, sem resíduo. Duas horas antes do TMF, os pacientes receberam 4 mg de loperamida via oral. Todas as colonoscopias foram realizadas por único pesquisador no setor de endoscopia do HC-UFMG com aparelho de duplo canal de trabalho (3,8 mm e 2,8 mm) e modelo Fujinon EC-530DM/DL. Os pacientes receberam analgésicos e hipnóticos para sedação profunda a cargo do anestesiologista. O aparelho foi introduzido até o ceco, com pouca insuflação e manobras de retificação para desfazer alças quando indicado. Ao delimitar o ceco, o máximo possível de ar foi aspirado e o paciente posicionado em decúbito lateral direito com objetivo de reter, por gravidade, o material no cólon direito e ceco. Após correto posicionamento, foi infundido no ceco todo o substrato fecal acrescido de 10 mL de ar injetado no canal de trabalho para aproveitamento de todo o material. O aparelho foi retirado sem insuflação de ar e sem avaliação da mucosa a fim de evitar distensão e estímulo peristáltico. Após o TMF, os pacientes foram encaminhados para a sala de recuperação anestésica onde permaneceram deitados em decúbito lateral direito por uma hora com objetivo de reter ao máximo o material transplantado. Os pacientes foram avaliados e liberados para casa no mesmo dia.

5.12. ACOMPANHAMENTO PÓS TMF

Após o transplante, os receptores foram acompanhados regularmente para avaliação da eficácia e ocorrência de possíveis eventos adversos a curto e longo prazo (Ficha de Acompanhamento do Receptor – Apêndice D). Na primeira semana os pacientes foram

assistidos diariamente, mediante contato telefônico, com abordagem de sintomas, investigação de possíveis complicações endoscópicas, eventos adversos e avaliação do tempo de resolução da diarreia. Na ocorrência de evento adverso grave ou queixas persistentes, os pacientes foram avaliados pessoalmente pelo pesquisador. Após a primeira semana, o seguimento foi periódico, por meio de contato telefônico, com oito semanas, três meses, seis meses e, posteriormente, anual. Os pacientes enviaram, após sete e 21 dias de transplante, amostra de fezes acondicionadas em frasco comum de exame, em caixa de isopor lacrada, sob refrigeração com gelo, para armazenamento no nosso laboratório. Os participantes foram orientados a entrar em contato com o pesquisador na suspeita de recidiva da infecção pelo *C. difficile* ou na presença de qualquer queixa ou evento adverso.

5.12.1. DEFINIÇÃO DE FALHA TERAPÊUTICA E RESOLUÇÃO DA ICD

Foi considerada falha terapêutica a ocorrência de diarreia nas primeiras oito semanas após o transplante, caracterizada por mais de três evacuações diárias, com fezes não formadas (Bristol 6 ou 7) por um período superior a 48h e confirmada laboratorialmente com a realização de GDH e cultura toxigência. A esses pacientes, foi oferecido um novo TMF com fezes de outro doador. A resolução de ICD foi definida com base em critérios clínicos, caracterizada pela ausência de diarreia e dor abdominal ao final de oito semanas de tratamento. A taxa de resolução pode ser primária se alcançada com apenas um TMF ou geral se forem necessários novos procedimentos em um período de oito semanas.

5.13. ANÁLISE ESTATÍSTICA

A análise estatística dos dados foi realizada utilizando-se o programa SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). As variáveis numéricas foram apresentadas como médias e desvio-padrão ou como medianas e seus valores mínimos e máximos quando a distribuição não foi gaussiana. As variáveis categóricas foram apresentadas em números absolutos e porcentagens.

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7. ARTIGO 1

Structuring a fecal microbiota transplantation center in a university hospital in Brazil

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ABSTRACT

Background: Fecal Microbiota Transplantation (FMT) is an important therapeutic option for recurrent or refractory *Clostridioides difficile* infection, being a safe and effective method. Initial results suggest that FMT also plays an important role in other conditions whose pathogenesis involves alteration of the intestinal microbiota. However, its systematized use is not widespread, especially in Brazil. In the last decade, multiple reports and several cases emerged using different protocols for FMT, without standardization of methods and with variable response rates. In Brazil, few isolated cases of FMT have been reported without the implantation of a Fecal Microbiota Transplantation Center (FMTC).

Objective: The main objective of this study is to describe the process of implanting a FMTC with a stool bank, in a Brazilian university hospital for treatment of recurrent and refractory *C. difficile* infection.

Methods: The center was structured within the criteria required by international organizations such as the Food and Drug Administration, the European Fecal Microbiota Transplant Group and in line with national epidemiological and regulatory aspects.

Results: A whole platform involved in structuring a transplant center with stool bank was established. The criteria for donor selection, processing and storage of samples, handling of recipients before and after the procedure, routes of administration, short and long-term follow-up of transplant patients were determined. Donor selection was conducted in three stages: pre-screening, clinical evaluation and laboratory screening. Most of the candidates were excluded in the first (75.4%) and second stage (72.7%). The main clinical exclusion criteria were: recent acute diarrhea, overweight (body mass index $\geq$ 25 kg/m²) and chronic gastrointestinal disorders. Four of the 134 candidates were selected after full screening, with a donor detection rate of 3%.

Conclusion: The implantation of a transplant center, unprecedented in our country, allows the access of patients with recurrent or refractory *C. difficile* infection to innovative, safe treatment, with a high success rate and little available in Brazil. Proper selection of qualified donors is vital in the process of implementing a FMTC. The rigorous clinical evaluation of donors allowed the rational use of resources. A transplant center enables treatment on demand, on a larger scale, less personalized, with more security and traceability. This protocol provides subsidies for conducting FMT in emerging countries.

Key words: Fecal microbiota transplantation, *Clostridioides difficile* infection, fecal microbiota transplantation center, stool bank.

INTRODUCTION

HUMAN GUT MICROBIOTA

The gut microbiota is one of the most complex systems in the human body. It consists of 10^{14} microorganisms and participates in important health functions such as digestion, immunity, vitamin synthesis, carbohydrate fermentation and bile metabolism [1,2]. When in balance, it plays an important role in resisting colonization of external pathogens. It develops from birth in a dynamic process, influenced by genetic and environmental factors such as way of delivery, breastfeeding, used medications, geographic location and lifestyle. From the age of three, microbiota becomes more stable, with less inter-individual variability, and remains so throughout adulthood. However, they do not maintain their composition in a fixed way and can change in response to environmental stimuli and factors of the host itself. It is considered as a metabolically active organ, composed of a number of organisms ten times greater than the number of cells in the human body and capable of exercising its function in the intestinal and extra-intestinal systems [1].

In human feces, enteric microorganisms correspond to almost half of solid portion [3]. In addition to bacteria that are present in number of 10^{11} per gram of dry feces, there are viruses in number of 10^{8} per gram of feces, archaea (10^{8} per gram of feces), colonocytes (10^{7} per gram of feces), fungi (10^{6} per gram of feces) and, yet protozoa, metabolites and genetic material, that compounds minority of it [3]. Each component acts synergistically to maintain local homeostasis between the microbiome and the host. Even bacterial DNA and dead cells have an immunostimulating and environmental balancing function.

Human microbiota knowledge has grown rapidly and has provided important discoveries. Through genetic sequencing techniques it is now possible to better characterize and study its changes in different situations and diseases [2]. The resulting disorder of its homeostasis, known as dysbiosis, has been related to several diseases pathogenesis, i.e. *Clostridioides difficile* infection (CDI), which has being the most studied affection. In this way, there is a progressive effort in the development of new therapies capable of modulating the microbiota, correcting dysbiosis and promoting advances in the treatment of these conditions. Among the possibilities of microbiota

modulation, the use of prebiotics, symbiotics, probiotics and, more recently, the Fecal Microbiota Transplantation (FMT) stands out.

FECAL MICROBIOTA TRANSPLANTATION

FMT is the procedure in which microbiota from healthy donors is transferred to the gastrointestinal tract of a sick person in order to repopulate their digestive tract, correcting underlying dysbiosis and promoting patient's recovery process. Unlike probiotics, the material introduced is composed of all diversity of species present in the donor's feces and capable of exercising its functions for an extended period [(4)]. FMT can be performed using various methods such as oral pills, nasogastric tubes, nasoenteric tubes and enemas or during endoscopic or colonoscopic procedures. There is, to date, no study that indicates the superiority of one over another [(5)].

HISTORY OF FECAL MICROBIOTA TRANSPLANTATION

Despite the enthusiasm in modern medicine, the use of healthy stools to treat sick people has been described since antiquity [(6)]. The first records on fecal transplantation date back to the fourth century in China. During the Dong Jin dynasty, physician Ge Hong first described, in his medical manual "*Zhou Hou Bei Ji Fang*", the ingestion of human fecal suspension for the treatment of food poisoning and severe diarrhea. Later, in the 16th century, the physician Li Shizhen documented in his traditional medicine book "*Ben Cao Gang Mu*" the use of dry stools, fermented fecal suspensions, and even fresh children's feces for the treatment of severe diarrhea, fever, vomiting, and intestinal constipation. For better acceptance, this treatment was called "yellow soup" or "golden syrup" [(6)].

During World War II, German soldiers in Africa (Afrika Korps) were told to use camel feces to treat bacterial dysentery. While many soldiers died of dysentery, the local population protected themselves by consuming fresh, warm camel feces at the first sign of illness. From this observation, Nazi scientists analyzed the feces and managed to isolate *Bacillus subtilis*, used later by the corporation with good results [(7)].

The first description of FMT in modern medicine dates back to 1958. Ben Eiseman successfully used fecal enemas to treat four patients with severe pseudomembranous colitis refractory to the use of antibiotics [8]. Even without microbiological confirmation, patients probably would have CDI.

In 2013, the first randomized clinical trial using FMT to treat recurrent CDI was published [9]. Patients were randomized to receive one of three therapies: (1) oral vancomycin 500mg every 6h for four days followed by intestinal lavage and subsequent FMT using nasoenteric tube; (2) therapy with isolated oral vancomycin (500mg 6/6h orally for 14 days) or (3) therapy with oral vancomycin plus intestinal lavage. The study had to be stopped after the initial analysis of the data, given the high effectiveness of FMT. Of the 16 patients in the FMT group, 13 (81%) had resolution of the diarrhea after the first infusion. The resolution of diarrhea in the group of vancomycin alone and vancomycin with intestinal lavage was 31% and 23%, respectively ($p < 0.001$). FMT was significantly more effective than standard treatment. From this randomized clinical trial, the effectiveness of FMT has been proven by other studies. Currently, FMT has become standard therapy for recurrent CDI and is currently under investigation for the treatment of other diseases.

INDICATIONS FOR FECAL MICROBIOTA TRANSPLANTATION

FMT has established itself as a promising therapy for CDI cases and has changed concepts about the management of these patients, specifically in the prevention of new recurrences. Based on case series, randomized clinical trials and meta-analyzes, the European Consensus on Fecal Microbiota Transplantation, Australian Consensus for Transplantation in Clinical Practice and American Society of Infectious Diseases decided to recommend FMT as treatment for refractory or recurrent CDI, especially from of the second recurrence episode [10-12]. There is not enough data to recommend it as treatment for the first episode, or as an adjunct to antibiotic therapy [12]. Marie Hocquart *et al*. have proposed that FMT should be considered a first-line treatment for severe cases of CDI, based on the significant reduction in three month mortality rate [13]. However, further studies are needed to make this proposal consensual.

There are several conditions in which FMT has been tested as a therapeutic option, although still on an experimental basis. Among them, it has been evaluated in inflammatory bowel disease, irritable bowel syndrome, obesity, peripheral insulin resistance, hepatobiliary disorders, hemato-oncological diseases, infections by resistant multidrug organisms and neurological syndromes [14]. However, the recurrent or refractory *C. difficile* infection represents the main indication for transplantation, considering the volume and strength of the evidences [11,12]. The effectiveness is around 90% in treating recurrent CDI and it is associated with few side effects, mostly mild and transient [9,15-18]. Beside this, it improves quality of life and is well accepted by patients [19]. Nevertheless, a minority of cases do not respond satisfactorily. Factors related to donors, recipients, and the procedure itself can contribute to treatment failure and require investigation. Elements that can negatively influence the result of the transplant include low stool volume, severe colitis, colitis with endoscopic evidence of pseudomembranes, concomitant use of other antibiotics, and hospitalization [11,12].

Ideally, it is necessary to structure transplantation centers and stool banks, capable of supplying material and carrying out the procedure in an agile and safe manner. Aware of the growing implantation of fecal transplantation centers, the *Food and Drug Administration* (FDA), in 2013, regulated FMT as a new treatment modality and established recommendations for the procedure [20]. In the same year, the Infectious Diseases Society of America (IDSA), American Society for Gastrointestinal Endoscopy (ASGE), North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN), American Gastroenterological Association (AGA) and American College of Gastroenterology (AGC) have issued consensual guidelines to regulate the screening and analysis of donor feces [21].

The current recommendation is that the FMT be performed in reference centers for the treatment of CDI, especially in hospitals with experience in the treatment of *C. difficile* and appropriate logistics [12,22]. The center must be composed of a multidisciplinary team led by a gastroenterologist, microbiologist or infectious disease physician, with appropriate scientific knowledge and experience with FMT. The director of the bank must ensure that the supply of fecal samples in clinical practice is only for CDI patients. For others indications, it is necessary perform research protocols and approve these by the local ethics committee under rigorous review [22]. The stool bank must also have the

participation of a biobank specialist capable of processing and storing the samples under standardized conditions and ensure compliance with quality standards required in the process [22].

RECURRENT OR REFRACTORY *C. DIFFICILE* INFECTION

The *Clostridium difficile* had its nomenclature recently changed and was renamed to *Clostridioides difficile* [23]. It represents the main pathogen responsible for diarrhea associated with human health care. It is a Gram-positive, spore-forming bacillus whose toxins cause gastrointestinal disease with a wide spectrum of severity. The clinical presentation ranges from mild diarrhea, pseudomembranous colitis to toxic megacolon, which can lead to death. Its incidence, severity and recurrence have increased worldwide over the last decades [24]. The indiscriminate use of antibiotics, especially quinolones, longevity of the population, and appearance of hypervirulent strains, responsible for major epidemics around the world, has contributed to the increase in morbidity and mortality associated with *C. difficile* infection [25]. The mortality rate in the United States increased from 1.5% to 6%, reaching 17% in periods of epidemic [24]. In cases of severe CDI, mortality is around 36-58% [26,27]. The infection is usually treated with antibiotics such as metronidazole, vancomycin or fidaxomicin, and has a recurrence rate of 25-30% [28]. More than that, patients who have subsequent relapses have a chance of developing a new recurrence of up to 60%, even when adequately treated with antibiotics [28]. The pathophysiological basis for this behavior is not restricted to the existence of antimicrobial resistance, but rather to the inability to reestablish a healthy intestinal microbiota that prevents the development of the new infection [29].

The mechanism involved in the recurrence is the re-exposure or reactivation of *C. difficile* spores in patients with deficient immune response associated with defect in the colonic epithelial barrier function and dysbiosis [30]. Patients with recurrent CDI have a reduced diversity of microbiota, usually secondary to prior antibiotics exposure used to treat others conditions or even to treat CDI [31]. The traditional antibiotic therapy used in CDI can generate a dysbiosis cycle by perpetuating the imbalance of the microbiota favoring a propitious environment to the proliferation of *C. difficile*. The exact

mechanism of action of FMT has not yet been fully elucidated, but the rationale for the transplant is that it is able to break the cycle of dysbiosis by introducing a new healthy microbiota, characteristically rich in diversity, capable to occupy the intestinal niche and prevent development of *C. difficile*. However, the microbiota is not the unique determinant for the therapeutic success of FMT. Study conducted by Ott *et al.,* in 2017 showed that transplantation with filtered and sterile fecal substrate, free of viable bacteria, is also effective in the treatment against recurrent CDI [(32)]. This finding suggests that non-bacterial substances such as proteins, antimicrobial compounds, metabolic products and oligonucleotides also contribute to the therapeutic effect of FMT.

C. DIFFICILE INFECTION SCENARIOAND FECAL MICROBIOTA TRANSPLANTATION IN BRAZIL

In Brazil, CDI is recognized as the main cause of nosocomial diarrhea related to the use of antibiotics [(33)]. Despite underreporting and few national epidemiological data, the number of records on isolation and characterization of *C. difficile* in our country is growing. Cançado *et al.,* in 2018, evaluated a cohort of adults admitted to a university hospital in Belo Horizonte who developed diarrhea after the use of antibiotics. The prevalence of CDI was 31.8% and was related to underlying comorbidities and the number of antibiotics used during hospitalization. Almost all of the toxigenic strains had the tcdA or tcdB genes. The main PCR ribotypes identified were 014/020 and 106. Binary toxin-producing strains not associated with ribotypes 027 and 078 have also been identified [(34)].

In the same year, in Brazil, the hypervirulent ribotype 027 *C. difficile* strain (NAP1 / 027) was isolated for the first time [(35)]. This strain was responsible for the increase in worldwide cases of CDI since 2000, including outbreaks in North America and Europe [(33)]. This more virulent ribotype had already been isolated in Australia, Asia, Central America and South America, but not yet in Brazil. However, despite the lack of reports of national outbreaks to date, the identification of the new hypervirulent ribotype 027 *C. difficile* strain and other strains producing binary toxin in the country raises the alert for

the need to optimize preventive measures, disseminate diagnostic methods and improve access to therapeutic measures, in particular, FMT.

Despite the advent of recurrent CDI cases in Brazil, FMT is still not a reality in Brazilian clinical practice. There are few reports of fecal transplantation in our country. So, as far as we know, only one study has been fully published in 2015, describing the experience of 12 patients with CDI undergoing transplantation in São Paulo city, with a success rate of 90% [36]. In all these cases, the procedure was not performed from a stool bank, a premise for structuring a fecal microbiota transplantation center based on international recommendations. In addition, there is a paucity of data on donor screening in emerging countries, especially in Brazil.

REGULATORY ASPECTS ABOUT FECAL MICROBIOTA TRANSPLANTATION AND STOOL BANK

In the United States, in May 2013, the FDA regulated FMT as a new therapy under investigation (*US Investigational New Drug*, IND) [20]. This decision was received with some concern by the medical community and by patients as it could limit access to a promising treatment modality. Later, in July 2013, the agency changed its previous declaration, releasing patients with recurrent CDI from the need to apply IND, since the informed consent form (IC) has been signed [21]. The term should explain that FMT is a treatment under investigation. It should address risks, benefits and alternative treatments. The donor and feces must pass screening tests carried out by the suitably qualified service provider [20]. The release did not include the other indications for transplant. To perform the FMT for other conditions, it is necessary for institutions to send an IND request.

In Europe, in December 2014, the European Commission considered the feces used in the transplant as a "combined product", composed of human cells and non-human components, such as the microbiome [37]. However, considering that the human component is not primarily responsible for the therapeutic response of FMT, the commission decided that the fecal substrate would not criteria to the guidelines of the European Tissue and Cells Directive. The competent authorities have therefore allowed the regulation to be managed at national implementing level. Member states would be

free to create specific regulatory structures for the transplantation in their territories and each stool bank must operate under the regulations of each country.

In Brazil, as well as in several countries in the world, there are no specific regulations for FMT. The International Consensus on Fecal Microbiota Transplant recommends that, in the absence of local guidelines, the transplant be performed under the aegis of a stool bank with a responsible scientific committee [22]. The bank must have a doctor to evaluate, select and recruit stool donors; microbiologist and /or pharmacist to coordinate all procedures related to stool processing and storage; a biobanks specialist to properly store fecal samples and a staff to ensure compliance with all steps. As it is a treatment under investigation, it is recommended that FMT be performed along the standards of a scientific study. According to the legislation that rules studies involving human beings, prior approval of the research protocol by the institution's Research Ethics Committee (REC) is required.

OBJECTIVES

GENERAL OBJECTIVE

To implement a fecal microbiota transplantation center with stool bank in a Brazilian university hospital for the treatment of patients with recurrent or refractory *C. difficile* infection, qualified for regional and national cases.

SPECIFIC OBJECTIVES

- To describe the process of structuring the fecal microbiota transplant center with a frozen stool bank.
- To define donor selection protocol.
- To establish procedure for preparation and storage of fecal samples.
- To determine procedures for administration of fecal substrate.
- To create a platform for future studies in the field of intestinal microbiota.

METHODS

INFRASTRUCTURE OF THE FECAL MICROBIOTA TRANSPLANTATION CENTER

The Fecal Microbiota Transplant Center (FMTC) was structured within Tumors and Tissues Bank of the Instituto Alfa de Gastroenterologia, Hospital das Clínicas, Universidade Federal de Minas Gerais (UFMG)/Empresa Brasileira de Serviços Hospitalares (EBSERH) (IAG-HC/UFMG), and developed within the required criteria approved by international organizations such as the FDA, European Fecal Microbiota Transplant Group and in accordance with national regulatory aspects. In this process, it had the partnership of the Bacteriosis Laboratory, Department of Preventive Veterinary Medicine, Escola de Veterinária UFMG and the Endoscopy Unit, IAG-HC/UFMG. FMTC is formed by a multidisciplinary team involving gastroenterologists, endoscopists, microbiologists, biomedicals, and pharmacists with experience in CDI, biobank, and donor and recipient procedures in human biological materials. FMTC and Tumors and Tissues Bank IAG-HC/UFMG have been approved by the Research Ethics Committee of the Federal University of Minas Gerais (CAAE 72755217.8.0000.5149 - Opinion 2.264.667 on September 8, 2017) and by National Commission for Research Ethics, respectively.

STUDY DESIGN

This work was divided into two stages. Firstly, a literature review was carried out on relevant aspects in the structuring of FMTC with a frozen stool bank. Articles linked to electronic banks PubMed, Lilacs, MEDLINE and Cochrane were searched and selected according to title, summary and relevance in the field of FMT. The main descriptors were Clostridium difficile (currently, renamed to Clostridioides difficile), recurrent Clostridium difficile infection, fecal microbiota transplantation, fecal transplantation, intestinal microbiota transplantation, donor selection, frozen stool and stool bank. The search was limited to studies published in English and Portuguese until September 2019. At the same time, meetings were held to outline protocols, carry out a standard operating procedure, review and analyze the measures already in place and define new

guidelines. Donors were selected and the methodology was used to prepare and store the fecal substrate. After acquiring the first samples, the functioning of the FMTC was publicized in state and national media such as TV news, radios, magazines and social networks of the Brazilian Federation of Gastroenterology and the Minas Gerais Gastroenterology Association.

The second stage of the study consisted of conducting a prospective, open, uncontrolled pilot study, in a single center, to evaluate the effectiveness of fecal microbiota transplantation in patients with recurrent or refractory CDI. Patients received the transplant from healthy donors selected in the first stage. Clinical and demographic variables, comorbidities, previous exposures to medications, severity of CDI and laboratory data of ten patients before and after treatment were evaluated. The aim was to report the initial experience with transplants and to determine the resolution rate and safety in short and long term. This stage is part of another study and is not included in this publication.

DONOR SELECTION

The donor selection was carried out prospectively in a three-step approach: (1) recruitment and pre-screening; (2) clinical assessment, and (3) laboratory screening with blood and stool tests (Figure 1). The selection criteria were conducted according to consensus recommendations among the protocols as well as Brazilian epidemiological specificities. The recommended criteria came from FMT Working Group, Amsterdam protocol, Australian protocol, the consensual guidelines in the letter from American societies to the FDA, European Consensus on FMT, International Consensus on Stool Banking for FMT and OpenBiome [9,12,22,38-41].

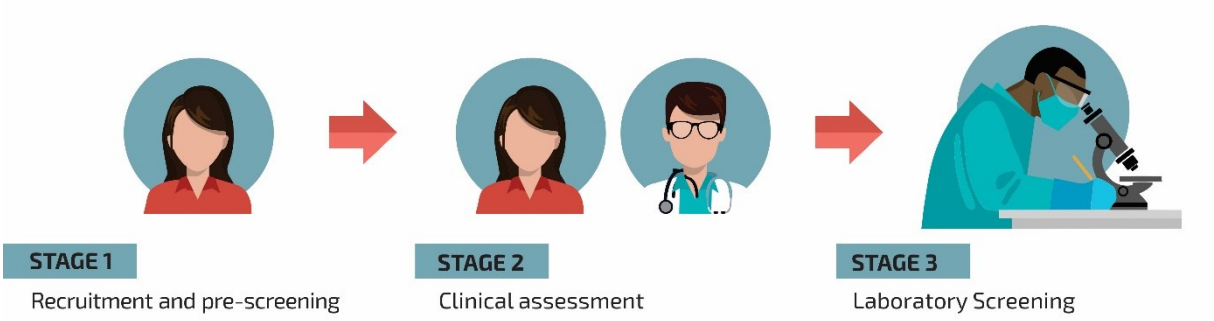

Figure 1. Steps in the process of selecting stool donors.

RECRUITMENT AND PRE-SCREENING

Volunteers were screened for eligibility assessment as fecal donors. Candidates received an invitation to voluntary participation and underwent a self-assessment that addressed four issues: (1) presence of a known disease; (2) body overweight; (3) recurrent digestive complaints and, (4) logistical unavailability for stool donation. The presence of at least one of these criteria made it impossible to continue the donor selection process. Those approved passed to the second stage as potential donors.

CLINICAL EVALUATION OF POTENTIAL DONORS

The clinical evaluation was performed by a single researcher and consisted of a complete medical interview, with details on health history, physical examination and analysis of inclusion and exclusion criteria. The candidates were submitted to a questionnaire similar to the one used in blood donation. Only donors approved at this stage were subjected to blood and stool tests.

LABORATORY EVALUATION OF POTENTIAL DONORS

Blood and stool tests for potential fecal donors are detailed in Figure 2 and Figure 3 respectively.

General blood tests		Serological tests
Complete blood count	Aspartate aminotransferase	Syphilis (VDRL)
C-reactive protein	Alanine aminotransferase	Hepatitis A, B and C (antibody test)
Urea, Creatinine	Gamma glutamyl transferase	HIV 1 and 2 (combined antibody and antigen test)
Sodium	Bilirubins	HTLV 1 and 2 (antibody test)
Potassium	Alkaline phosphatase	Chagas disease (antibody test with two combined methods: hemagglutination and indirect immunofluorescence)
Chlorine	Albumin	Schistosomiasis (antibody research)
Magnesium	Prothrombin time	
Calcium	Partial thromboplastin time	
Glucose	Total cholesterol and fractions	
25-hydroxyvitamin D	Triglycerides	
Folic acid	Thyroid-stimulating hormone	
Vitamin B12	Free T4	

VDRL, venereal disease research laboratory; HIV, human immunodeficiency virus; HTLV, human T-lymphotropic virus

Figure 2. Blood testing for potential fecal donors

Bacterial tests	Parasitic tests	Viral tests
C. difficile (GDH and toxigenic culture)	Microscopy for eggs and parasites in three serial samples	Norovirus (PCR)
Enteric pathogens: *Salmonella* sp., *Shigella* sp., *Campylobacter* sp., *Vibrio cholerae, Yersinia* sp. (culture)	*Giardia lamblia* (microscopy and antigen)	Rotavirus (PCR)
Escherichia coli O157 (isolation and PCR)	*Strongyloides stercoralis* (microscopy and Baermann-Moraes method)	Coronavirus (PCR)
Salmonella sp. (isolation and PCR)	*Entamoeba histolytica* (microscopy and antigen)	
Clostridium perfringens (isolation and PCR)	*Schistossoma mansoni* (microscopy)	
Campylobacter sp. (PCR)	Isospora (microscopy)	
MRSA, VRE, ESBL-producing enterobacteria and carbapenemase-producing enterobacteria (culture)	Microscoridia (microscopy)	

GDH, glutamate dehydrogenase; PCR, polymerase chain reaction; MRSA, methicillin-resistant *Staphylococcus aureus;* VRE, vancomycin-resistant enterococci; ESBL, expanded-spectrum beta-lactamase

Figure 3. Stool testing for potential fecal donors

DONOR INCLUSION CRITERIA

Adult individuals, of both sexes, related or not to the recipient, aged between 18 and 50 years old and who agreed to the process of selection and donation of feces by signing the informed consent form.

DONOR EXCLUSION CRITERIA

Donor exclusion criteria are detailed in Figure 4.

- Fever of unknown origin or fever in the last 2 weeks or active infection not controlled during donation.
- Exposure to antibiotics, immunosuppressants or chemotherapy in the last 3 months.
- Active communicable disease (HIV, hepatitis A, B or C).
- Known exposure or previous history of HIV 1 and 2, hepatitis B, hepatitis C, syphilis, HTLV 1 and 2, malaria, Chagas disease, tuberculosis, cutaneous and mucous herpes.
- History of gastrointestinal complaints or diseases, including inflammatory bowel disease, irritable bowel syndrome, celiac disease, diarrhea or chronic intestinal constipation, gastrointestinal malignancies, polypoid syndromes, excess of gas, flatulence or major gastrointestinal surgical procedures.
- Previous history of organ and tissue transplantation (including cornea).
- History of blood transfusion in the last 6 months.
- History of biological accident with sharp-edged objects in the last 6 months.
- Recent history (last 2 months) of vaccination with live attenuated virus.
- History of autoimmune, atopic diseases or ongoing immunomodulatory therapy.
- Risk factors for Creutzfeldt-Jacob disease (previous or family history, graft recipients such as corneal transplant, previous use of cadaveric pituitary hormones, previous use of bovine insulin, nosocomial exposure, people who remained in the United Kingdom and/or Ireland for more than three months between 1980 and 1996 or who have stayed for more than five years, consecutive or intermittent, in Europe after 1980 to the present day).
- Health professionals exposed to the risk of transmitting infectious diseases or the risk of being carriers of multidrug-resistant organisms.
- Professionals who work with animals, at risk of transmission of zoonoses.
- High-risk sexual behavior (sexual contact with anonymous people, sexual contact with sex workers, drug use before sexual intercourse, sexual contact with individuals with HIV or viral hepatitis, man who has sex with man, relationship with bisexual man, multiple partners sexual and sex workers).
- New sexual contact in the last 12 months.
- Previous history of sexually transmitted disease.
- Use of intravenous or inhaled illicit drugs.
- Recent history of hospitalization (for more than 2 days in the last 3 months), incarceration or stay in long-term health institutions.

- Implant of piercing, earrings, tattoos, or acupuncture in the last 6 months.
- Recent history of hematochezia or other bleeding from the gastrointestinal tract (last 2 months).
- Recent acute diarrheal disease in the last 6 months.
- Overweight and obesity defined by the World Health Organization as a body mass index (BMI) greater than or equal to 25 and 30 kg/m² respectively.
- Moderate to severe malnutrition.
- Diabetes mellitus, metabolic syndrome.
- Psychiatric disorders; chronic pain syndromes (fibromyalgia, chronic fatigue) or neurological syndromes.
- History of malignant neoplasms.
- Chronic use of proton pump inhibitors (for at least 3 months).
- Family history of polypoid syndrome or premature colorectal cancer (under 50 years old) in a first-degree relative.
- Detection of any changes in blood tests or stool tests.

Figure 4. Diseases or conditions that permanently or transitively excluded a potential donor

FECAL SUBSTRATE COLLECTION

Donors were requested to do weekly collections in the first month and every 15 days for the next three months. At the time of feces collection, donors were evaluated on their health conditions since the last screening. Telephone contact was made with each donation to address the following risk factors: (1) development of diarrhea; (2) presence of any disease or complaint; (3) use of antibiotics or new drugs; (4) new sexual contact. Donors with symptoms of active infection or one of the risk factors mentioned above were temporarily excluded. After the period defined in the exclusion criteria (six months for diarrhea, three months for antibiotics, 12 months for new sexual contact), the candidate was summoned and, if they agreed, submitted to a new screening process.

The collections were performed during four months after approval in the donor selection process. After this period, the donor was invited to remain in the program. For this, it was necessary to undergo a new screening with a complete clinical evaluation, blood and fecal exams.

The donated feces were collected in routine examination vials (50 mL / 50 g of feces) according to the standard stool collection procedure, on a clean and dry plastic surface and at home. The material identified with the donor's name, date and time of collection

was sent to the FMTC laboratory within two hours. The professional responsible for receiving and preparing the material wore an apron, gloves, mask, and facial protection during handling.

SAMPLE PROCESSING AND STORAGE

The stool preparation procedure for FMT was carried out in an appropriate and exclusive space (biological risk level 2). After weighing, feces were transferred to a container containing 0.9% non-bacteriostatic saline solution in the proportion of 50 g of feces for each 250 mL of saline. The mixture was homogenized manually for two to five minutes. The suspension was transferred carefully to another container previously prepared with a funnel gauze filter, five overlapping open gauzes and elastic for fixation. The suspension was filtered through gauze twice, in order to remove dietary fibers and coarse dirt that could obstruct the colonoscope's working channel. After filtration, glycerol was added for cryoprotection (preventing the formation of crystals) to get final concentration of 10%. The fecal suspensions were then placed in plastic containers with lids and stored in an ultra-freezer at -80 °C until use. The viability time established from preparation to administration was six months.

DEFROSTING AND PREPARING MATERIAL FOR INFUSION

On the day of the transplant, 250 to 300 mL of fecal suspension was removed from the ultra-freezer and thawed. The aliquots were thawed at room temperature, at 4ºC and/or in a water bath at 37 °C. The method chosen for thawing varied according to the time of the procedure. After complete defrosting, the material was transferred to 60mL syringes, without a needle, with the aid of a 14 French gauge aspiration probe. The syringes were sealed, identified and packed in their own container (stainless steel vat) for transportation in a styrofoam box containing gel ice. Once thawed, fecal suspension should be used within six hours if at room temperature or up to eight hours under refrigeration. The samples could not be frozen again if they were not used.

RECEPTORS INCLUSION CRITERIA

Patients with recurrent or refractory CDI infection who agreed to participate in the research by completing the informed consent form were included.

DEFINITION OF RECURRENT AND REFRACTORY C. DIFFICILE INFECTION

Recurrent infection was defined as the development of a new CDI within eight weeks of a previous episode adequately treated, in which there was an initial resolution of symptoms. Recurrence was characterized by the presence of diarrhea, with more than three daily excrement, with unformed feces (Bristol 6 or 7), during minimum period of time of 48 hours, and positive laboratory results using GDH ECO Test - TR.0032 (Eco Diagnóstica, Minas Gerais, Brazil) confirmed by toxigenic culture [42]. Refractory infection was defined as persistent infection, with no improvement in symptoms, despite antimicrobial treatment with oral vancomycin for at least five days.

INTENSITY OF C. DIFFICILE INFECTION

- Complicated CDI: infection complicated with toxic megacolon, peritonitis, hemodynamic instability, respiratory failure or need for surgical approach.

- Severe CDI: one of the following criteria (bloody diarrhea, pseudomembranous colitis, adynamic ileus, severe abdominal pain, fever with an axillary temperature greater than 38.9°C, serum albumin below 2.5 g/dL, higher global leukocyte count at 20.000 cells/mm³, acute renal failure).

- Mild to moderate CDI: diarrhea without additional criteria that characterize a severe or complicated condition [43].

RECEPTORS EXCLUSION CRITERIA

- Pregnancy.
- Septic shock defined as: sepsis requiring a vasopressor to raise mean arterial pressure ≥ 65 mmHg and lactate > 2 mmol/L despite adequate volume expansion.
- Life expectancy less than three months.

- Patients unable to undergo colonoscopy.
- Inability to complete the informed consent form (own patient or related family member).
- Absence of criteria for recurrent or refractory C. difficile infection.
- Those who refuse or give up participating in the research.

MANAGEMENT OF RECEPTORS BEFORE THE FMT

The transplant candidates underwent a medical interview to characterize the clinical history. The following variables were assessed: pre-existing comorbidities, Charlson score, medications in use, history of allergies, duration of symptoms, number of bowel movements per day, stool shape according to the Bristol scale, and previous treatments for CDI [44,45]. A stool sample was collected for diagnostic confirmation and storage. The diagnosis was confirmed through the GDH test and toxigenic culture. Patients have received 125mg oral vancomycin, 6/6 h, for 10 to 14 days before FMT in order to reduce the intestinal population of C. difficile. The vancomycin was interrupted with an interval of 12 to 24 hours before the procedure. Patient and family members were instructed on how to disinfect home bathrooms with a sporicidal agent. It was recommended to use 0.525% sodium hypochlorite (9:1 water solution with bleach) for cleaning the bathroom, door handles, toilet bowl, sinks and taps on the day of the procedure. The patient was instructed not to use the bathroom until the FMT was performed.

BOWEL PREPARATION AND ROUTE OF ADMINISTRATION

Patients received usual intestinal preparation for colonoscopy with polyethylene glycol (PEG 4000), dimethicone and bisacodyl. Osmotic laxative was taken in two doses: 120g of PEG was diluted in one liter of water and administered orally in the night before and in the morning of the procedure. A 15mL vial of 75mg/mL dimethicone was diluted in the second dose of the preparation. Four bisacodyl 5mg tablets and a diet without residue were used the day before. The recommended fasting was 8 hours for small meals and 2 hours for clear liquids, without residue. Two hours before FMT, patients received 4mg of loperamide orally. All colonoscopies were performed by a single

researcher in the Endoscopy Department of HC-UFMG with a double working channel device (Fujinon EC-530DM/DL). Patients received analgesics and hypnotics for deep sedation under anesthesiologist assistance. The device was inserted up to the cecum, with little insufflation and maneuvers to undo handles when indicated. When delimiting the cecum, as much air as possible was aspirated and the patient was positioned in the right lateral decubitus position with the objective of retaining, by gravity, the material in the right colon and cecum. After correct positioning, the fecal substrate was infused into the caecum plus 10mL of air injected into the working channel to use all the material. The device was removed without air insufflation and without assessment of the mucosa, in order to avoid distension and peristaltic stimulation. After the FMT, patients were referred to the post-anesthetic recovery room where they remained lying in the right lateral decubitus position for one hour in order to retain the transplanted material as much as possible. Patients were evaluated and released home on the same day. The stages of the donation process, sample preparation and administration are shown in Figure 5.

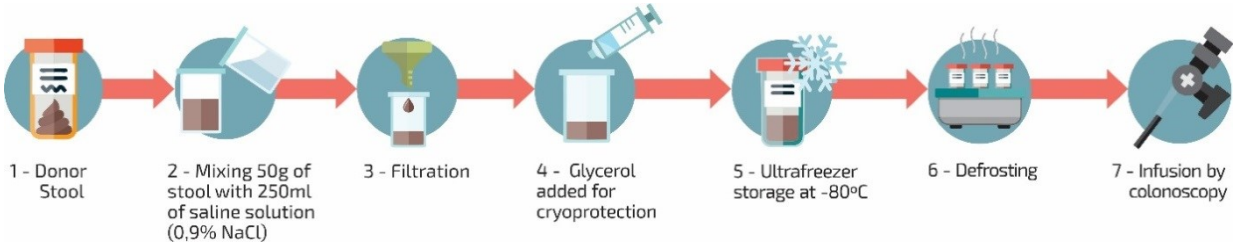

Figure 5. Phases of fecal microbiota transplantation with stool bank

POST MONITORING FMT

After transplant, receivers were monitored regularly to assess the effectiveness and occurrence of possible adverse events in the short and long term. In the first week, patients were assisted daily, by telephone contact, with a symptom approach, investigation of possible endoscopic complications, adverse events and evaluation of diarrhea resolution. If serious side effects were suspected or persistent complaints were registered, patients were personally assessed by the researcher. After the first week, the follow-up was done within eight weeks, three months, six months and, subsequently, annually. The patients sent, after seven and 21 days of transplantation, a sample of feces

stored in a common examination bottle, in a sealed styrofoam box, under refrigeration, to be stored in our laboratory. Participants were instructed to contact the researcher on suspicion of recurrence of C. difficile infection or in the presence of any complaint or adverse event.

DEFINITION AND CLASSIFICATION OF ADVERSE EVENTS

Adverse events were defined as any undesired occurrence after FMT, without the need for an exact causal relationship. Symptoms, disease onset or laboratory findings were considered.

They were classified according to severity in:

- minor events: mild symptoms, such as abdominal discomfort, diarrhea, constipation, flatulence, borborygmus, abdominal bloating, nausea, vomiting and fever with spontaneous resolution;
- major events: endoscopic complications (perforation, bleeding), complications related to sedation (bronchoaspiration), transmission of pathogens, exacerbation of inflammatory bowel disease, occurrence of infection (peritonitis, pneumonia), need for hospitalization, temporary or permanent functional disability or death.

Regarding the time of occurrence, they were classified as:

- short term: occurrence within one month after the FMT;
- medium term: between one month and one year;
- long term: after one year.

As for causality, they were classified into:

- definitely related: there was a reasonable temporal sequence, with an expected response pattern and not explained by another hypothesis;
- probably related: there was a reasonable time sequence, with an expected response pattern and unlikely to be explained by the patient's characteristics or other interventions;
- possibly related: despite the temporal relationship, it is possible that it is caused by factors other than transplantation;
- unrelated: event that is certainly unrelated to treatment.

DEFINITION OF THERAPEUTIC FAILURE AND CDI RESOLUTION

The occurrence of diarrhea in the first eight weeks after transplantation was considered a therapeutic failure, characterized by more than three daily bowel movements, with unformed stools (Bristol 6 or 7) for a period longer than 48 hours and CDI confirmed by GDH test and toxigenic culture. These patients were offered a new FMT with feces from another donor. The resolution of CDI was defined based on clinical criteria, characterized by the absence of diarrhea, leukocytosis and abdominal pain at the end of eight weeks of treatment. The resolution rate can be primary if achieved with just one FMT, or it can be general if new procedures are needed to achieve therapeutic success within an eight-week period.

STATISTICAL ANALYSIS

Statistical analysis of the data was performed using the SPSS program (IBM Corp. Released 2013; IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). The numerical variables were presented as means and standard deviation or as medians and range when the distribution was not Gaussian. Categorical variables were presented in absolute numbers and percentages.

RESULTS

Over a five-month period (September 2017 to February 2018) a total of 134 candidates were recruited to participate in the donor selection process. After self-assessment, candidates who met at least one of the exclusion criteria were eliminated from the screening process. Of the 134 possible candidates for donation, only 33 (24.6%) qualified as potential donors and went on to the second stage.

This subgroup underwent clinical evaluation that includes medical interview and detailed physical examination. The characteristics of potential donors are shown in Table 1. It was compound by 20 women and 13 men, with an average age of 32.9 ± 9.2 years, average body weight of 69 ± 12.8 Kg, height of 1.7 ± 0,10 meters and BMI of

23.8 ± 3.40 Kg/m². Twenty-six candidates (78.8%) were not related to possible receivers.

Table 1. Characteristics of the 33 potential donors.

Variable	N (%) / Mean ± SD
Gender	
Male	13 (39.4%)
Female	20 (60.6%)
Age (years)	32.9 (18 - 50)
Weight (kg)	69 (43.7 - 92.5)
Height (m)	1.7 (1.5 - 1.9)
Body Mass Index - BMI (Kg / m²)	23.7 (17.5 - 32.3)
Relationship with receiver	
Relatives	7 (21.2%)
(Not related)	26 (78.8%)
Pattern of alcohol consumption	
Abstinence	18 (54.5%)
Low consumption (up to 2 doses / day)	15 (45.5%)
Smoking	None
Regular use of medicines	10 (30.3%)
Normal intestinal habit	25 (75.7%)
Bristol scale	
Type 1	2 (6.0%)
Type 2	6 (18.2%)
Type 3	19 (57.6%)
Type 4	6 (18.2%)

More than half were married (54.5%), with a stable relationship. No donor candidate reported problems with alcoholism and 15 (45.5%) reported social consumption, with a maximum daily intake of 20 g of ethanol for men and 14g for women. None were smokers and 69.7% did not use medications continuously. The majority (75.8%) had a regular bowel habit, with normal stools, classified as type 3 and 4 by the Bristol scale.

Based on feature described, 24 (72.7%) candidates were excluded for presenting any clinical contraindication. The main contraindications found in the second stage were:

occurrence of acute diarrhea in the last six months, overweight (BMI ≥ 25 Kg/m²) and gastrointestinal disorders (constipation, irritable bowel syndrome, dyspeptic complaints and food intolerances). Half of the participants presented more than one contraindication. One candidate in particular was excluded for five reasons: acute diarrhea in the last six months, use of antibiotics in the last three months, tattooing in the last six months, risky sexual behavior and use of illicit drugs.

Nine participants continued the process and underwent blood and stool tests. Five (55.6%) were excluded based on the following reasons: presence of occult blood in the feces, presence of free-living protozoa (*Endolimax nana* and *Entamoeba coli*), positive test for *Salmonella* sp. and *Isospora belli*. Of a total of 33 potential donors, only four (12.1%) were selected after complete screening. The overall donor detection rate, considering all recruited candidates, was 3%. The exclusion criteria and results of donor selection are shown in Table 2 and Figure 6.

The candidate excluded due to the presence of occult blood in the feces was a female volunteer, 40 years old, married, nulligest and unrelated to the receptor. After extensive investigation a deep endometriosis of the cecum was found and surgically removed. Of the four qualified donors, three were relatives of a possible receptor who was being assessed for eligibility for FMT. However, the patient responded favorably to treatment with oral vancomycin for eight weeks and was not submitted to FMT. The three related donors performed only one stool collection each and lost follow-up after discarding the need for fecal transplant.

The remaining donor performed three collections in the period between March and December 2018. She evolved with recurrent abdominal pain and was referred for evaluation at the Gastroenterology outpatient clinic of HC-UFMG. However, after an initial evaluation, she showed clinical improvement and lost follow-up, making no further donations.

Table 2. Exclusion criteria for 29 potential donors.

Candidate	Gender	Age (years)	Exclusion Criteria
1. NCRG	F	27	Student of veterinary medicine
2. KOC	F	28	Acute diarrhea in the last six months
3. LFFR	M	21	Overweight
4. CLFJ	F	37	Acute diarrhea in the last six months
5. LGFR	M	21	Overweight
6. ECF	F	41	Overweight
7. JJGB	M	35	Genital herpes
8. CCC	F	36	Intermittent diarrhea and lactose intolerance
9. EOSS	F	42	Overweight, depressive disorder
10. ECAM	F	38	Acute diarrhea in the last six months
11. GMM	F	39	Acute diarrhea in the last six months
12. RMM	M	31	Overweight
13. NCR	F	41	Genital herpes, overweight, depressive disorder
14. AVAS	F	21	Angioedema, recent vaccine with live attenuated virus
15. LRS	M	23	Irritable bowel syndrome, acute diarrhea in the last six months, recent antibiotic use
16. MICR	F	45	Obesity, depressive disorder
17. ACR	M	47	Ankylosing spondylitis, acute diarrhea in the last six months, overweight
18. WSLR	F	50	Chronic constipation, obesity, functional dyspepsia, chronic use of proton pump inhibitor
19. DMA	M	28	Vitiligo
20. DL	M	28	Genital warts
21. LR	M	38	Acute diarrhea in the last six months
22. PNA	F	22	Acute diarrhea in the past six months, chronic constipation, depressive disorder, recent antibiotic use
23. IC	F	18	Splenomegaly on physical examination
24. MAP	M	25	Risky sexual behavior, use of illicit drugs, tattooing in the last six months, acute diarrhea in the last six months, recent use of antibiotics
25. HRVC	M	34	Positive for *Isospora belli* and *Endolimax nana*
26. CNMCG	F	35	Positive for *Entamoeba coli*
27. MAV	F	48	Positive for *Blastocystis hominis*
28. RSO	M	41	Positive for *Salmonella* sp.
29. KJVS	F	40	Presence of occult blood in the stool

F, Female; M, Male

After that period, 21 new candidates with an appropriate clinical profile were recruited. One of them, during the protocol examinations, had presence of *Entamoeba coli* cysts detected in a stool sample. We then opted for treatment with secnidazole 2g, orally, single dose and new stool collection, in three serial samples, after 15 days of treatment. The same was offered to the candidate who had been excluded in the first selection process. Control tests from both donors were negative for free-living protozoa and candidates were accepted as donors.

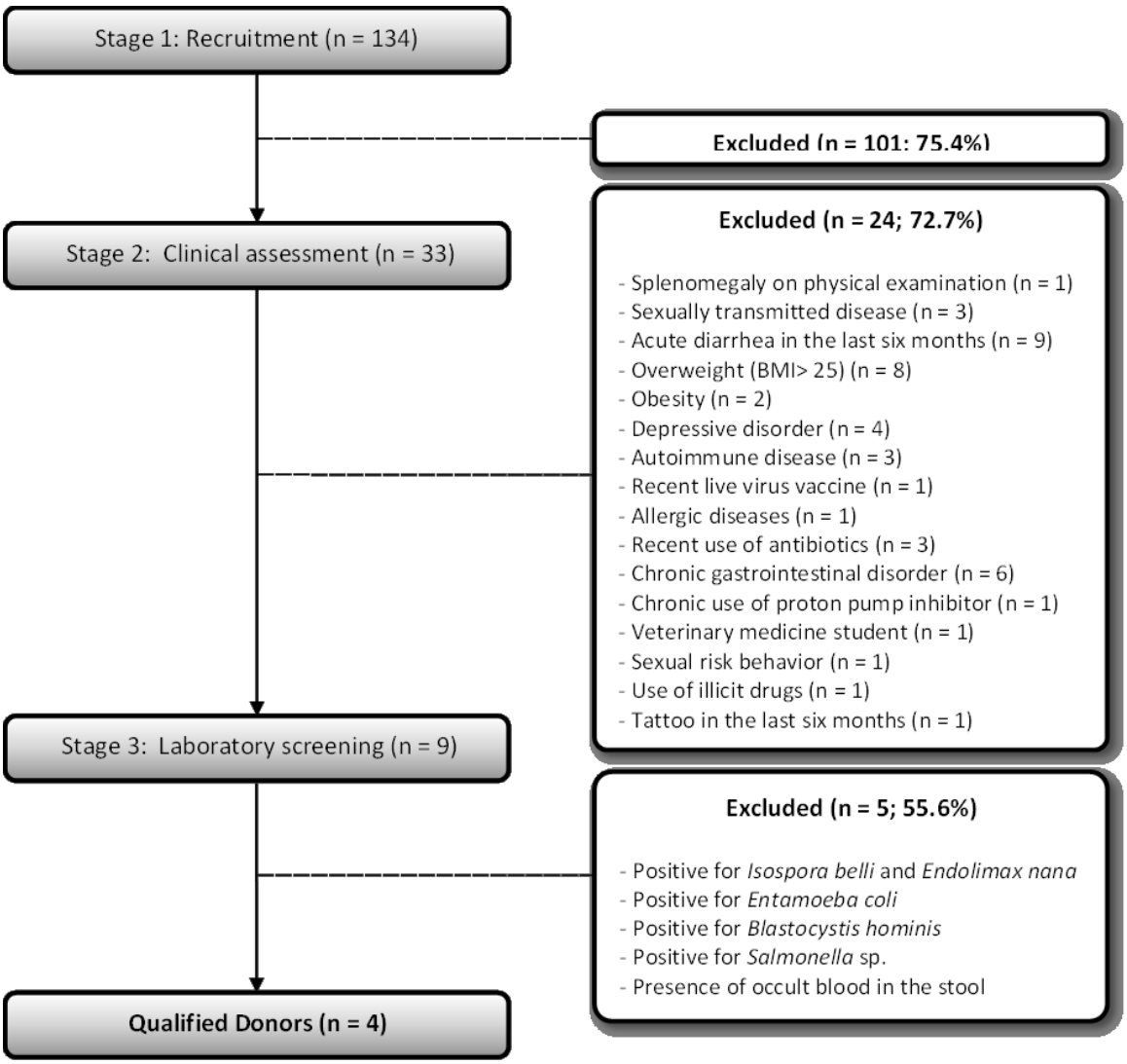

Figure 6. Three-step approach to donor selection process and main contraindications detected

Donations and stool preparation took place according to the standard operating procedure developed and approved by the transplantation center. No changes were identified that prevented the collection of feces on the day of collection. In total, 16 donations were made between October 2017 and March 2020. Donors collected the material in the morning in a clean, dry plastic container. The material was packed in a styrofoam box, sealed, identified with name, date, collection time and sent to the FMTC laboratory. The first donation arrived after 1h40min of the collection. It remained refrigerated between 2ºC and 8ºC and was processed the next day, with a total time between collection and storage of 29h. All other donations had a median time between collection and storage of 4h30min (Table 3). The median fecal weight donated was 71.3 g and the volume generated was 362.2 mL. Eleven transplants were performed using 280 mL (250 – 300 mL) of fecal substrate in each procedure. The time between the beginning of defrost and infusion of the fecal substrate was less than six hours.

Table 3. Characteristics of stool donations and sample preparation

Variable	**N / Mean ± SD**
Total number of donations	16
Donor 1	1
Donor 2	1
Donor 3	1
Donor 4	3
Donor 5	4
Donor 6	6
Number of bottles per collection	3 ± 1
Weight of samples collected (g)	71.3 ± 29.1
Volume generated at each donation (mL)	362.2 ± 168.7
Time to storage	4h30min ± 73min
Defrost time	2h50min ± 63min
Time between defrost and beginning of infusion	1h18min ± 48min
Volume prepared for infusion (mL)	280 ± 22.9

DISCUSSION

DONOR SCREENING

For the full functioning of a FMTC, it is necessary to recruit a large number of potential donors and select, among them, only healthy ones. Finding donors is a major challenge for structuring a transplant center, given the rigor of the selection process and the lack of evidence-based guidelines. Current criteria for donor screening and selection come mainly from regulatory institutions guidelines or scientific societies, based on expert opinion. There are no randomized clinical trials on donor characteristics and transplant effectiveness. In addition, the concept of healthy microbiota is still under construction. Previous studies used as a parameter of healthy microbiota that from healthy donors, with normal weight for height and without chronic diseases, allergies, high-risk behavior for sexually transmitted infections, family history of malignancy or chronic medication use [9,12,46].

Donor selection recommendations require candidates to undergo screening similar to that used for blood donations. The questionnaire must be able to identify risk factors or diseases with risk of transmission through the FMT. Some protocols also require monitoring at the time of donation to track changes in the interval between application of the questionnaire and donation of feces [9,39].

As general guidelines, only healthy adults, without chronic or acute illnesses, are qualified as stool donors. The selection is based on the identification and exclusion of candidates who present unfavorable conditions such as: (1) infectious diseases transmitted by blood and possibly by FMT, (2) conditions for which there is a reasonable possibility of transmission, (3) morbidities in which the microbiota plays a role in pathogenesis, (4) situations that increase the likelihood of transmitting infections from multidrug-resistant organisms (MDRO).

Almost all studies on FMT emphasize caution with screening donors given the risk of transferring infectious pathogens and the potential risk of transmitting phenotypes associated with dysbiosis, such as atherosclerosis, colorectal neoplasia, obesity, among others [47-51]. The caution is similar to that used in blood centers, especially after the HIV epidemic in the 1980s and cases of transmission of Hepatitis C by blood

transfusion, before its discovery in 1989. The precautionary principle aims to ensure that the risk of transmitting infectious diseases is reduced to very low levels.

Stool donation should be voluntary. Donors should be informed about all stages of the screening process, about conducting a medical interview, physical examination, blood and stool tests and the measures guaranteed to keep the process confidential. They must be informed of the potential risks and benefits of the donation and agree to the informed consent form. Candidates must be at least 18 years old and commit to providing honest answers, informing the transplant center if they become ill. They must allow their data and fecal samples to be stored and tested in the future if serious adverse events occur. It is also ensured that the donor can withdraw from the process at any time without any harm. All informed consent form must be stored for at least 10 years.

Despite seeming innocuous, the selection process presents considerable risks as exemplified in the present study. The discovery of an indolent disease, in an asymptomatic phase, should be considered before the investigation begins. The risk and emotional effect with false positive results should also be discussed with the candidate. On the other hand, the selection process can generate some benefits for the donor, such as the opportunity for a broad and non-invasive assessment of their health status and the possibility of the exercise of altruistic gesture, inherent to donations.

CLINICAL CRITERIA FOR DONOR SELECTION

Meta-analysis of 168 clinical studies on FMT, of which 108 were on CDI, shows that the exclusion criteria are heterogeneous in more than 50% of the studies [52]. The main clinical criteria for donor exclusion were recent use of antibiotics followed by gastrointestinal disorders and a history of malignancy. Gastrointestinal disorders include irritable bowel syndrome, inflammatory bowel disease, constipation and chronic diarrhea. Other common criteria were high-risk sexual behavior, tattoos and *piercings*. Less than half of the studies excluded diseases such as metabolic syndrome, psychiatric, neurological disease or diabetes mellitus.

Most candidates are excluded from the process after careful medical evaluation. Data from OpenBiome, a large American stool bank, shows that 67.8% of applicants who remained in the program after a medical interview were excluded due to clinical criteria,

with emphasis on the presence of asthma, atopy, medication use, psychiatric conditions and a history of infectious diseases [22]. A similar data was found in an Australian bank, excluding approximately 50% of donors after a clinical questionnaire [53]. In the present study, clinical criteria were able to exclude 72.7% of potential donors and reduce expenses with blood and stool tests in a more restricted group. Developing screening tools like ours is extremely important in emerging countries, where the good management of financial resources has a great impact on population health.

Comparison between the first transplant protocols and the latest International Consensus on FMT shows that the clinical criteria used in our institution are more rigorous than those used in first studies and similar to those currently recommended. This protocol stands out for having selected donors who are not overweight, with a narrower age range and a longer microbiota recovery interval after an episode of acute diarrhea (six months *versus* two months). In addition, it adds criteria not used in the first studies, such as a history of psychiatric disorders, chronic use of proton pump inhibitors, diabetes mellitus, neurological disorders, chronic painful syndrome and recent use of live attenuated virus vaccine (Table 4).

There is no consensus on the ideal age of donors. In order to apply the informed consent form, the applicant must be at least 18 years old in Brazil. In addition, the intestinal microbiota of children has less diversity in the first years of life and develops progressively over the years [54]. This finding explains, in part, the scarcity of children as donors in the large studies on FMT. Donor ages range from 18 to 50, 60 and 65 years old [9,39-41]. Increasing age has been associated with changes in the intestinal microbiota, which justifies a preference for donations from people under 50 years or those under 60 and adequately screened for colorectal cancer [22]. At this point, some studies recommend testing for fecal occult blood. However, only a minority of them (3.1%) screened their donors [52]. Male and female donors are equally eligible, even with a higher prevalence of autoimmune diseases and functional disorders in women [52]. On the other hand, male donors are more likely to provide greater fecal mass [52].

Many non-infectious disorders have been linked to dysbiosis such as neurodegenerative diseases, psychiatric disorders, autoimmune diseases, metabolic syndromes, hepatobiliary and chronic intestinal disorders [55]. However, the causal relationship is not as clear as in CDI. Most specialists agree to exclude from the selection process

individuals who have disorders related to dysbiosis due to the theoretical risk of long-term transmission [15,21,22]. The lack of evidence from long-term follow-up justifies this cautious attitude.

Some studies report a body mass index (BMI) greater than 30 kg/m² as exclusion criteria. Experiments in animal models show that microbiota receptors from obese mice increased their adiposity after transplantation even with maintenance of a standardized diet [56]. In addition, among the studies that did not report the donor's BMI, three receptors showed weight gain after FMT, with no other apparent cause [57]. Thus, it was decided to be more rigorous excluding candidates with a BMI above 25 kg/m² in the present study. Furthermore, there was an additional gain in eliminating candidates with possible disorders associated with overweight such as insulin resistance and metabolic syndrome.

Personal history of cancer should also be investigated in donors and recognized as a contraindication. However, candidates with a history of non-malignant skin cancer who have undergone appropriate treatment may be eligible [22].

Many stool banks exclude health professionals from the screening process due to the occupational risk of biological accidents and the possibility of colonization by multidrug-resistant organisms (MDRO). A systematic review assessed the risk of occupational colonization by MDRO in employees of hospitals and geriatric centers. Despite the methodological limitations and heterogeneity of the studies, the prevalence of colonized professionals was 2.6-48.5% for pathogens producing expanded spectrum beta-lactamase, 0-9.6% for vancomycin-resistant enterococci and 0.9-14.5% for methicillin-resistant *Staphylococcus aureus*. The real impact of transmission and consequent infection related to health care has not been measured [58]. In Brazil, a study with 294 oncology hospital workers identified colonization in the oral cavity by *Enterobacteriaceae* in only 18.7%, less than half of which was due to multidrug-resistant germs. The study did not identify colonization by ESBL or *Klebsiella pneumoniae* producer of carbapenemase [59].

Table 4. Clinical exclusion criteria for stool donation

Exclusion Criteria	FMT workgroup recommendations (2011)	Amsterdam Protocol (2013)	International Consensus on Stool Banking (2019)	**Protocol IAG HC-UFMG**
Age	—	< 18 and > 60 years	> 60 years or > 50 years without CaCR screening	< 18 and > 50 years
BMI	—	—	> 30 Kg / m²	> 25 Kg / m²
Risk factor or history of transmissible disease	✓	✓	✓	✓
ATB use in the last 3 months	Excluded if < 3 months	✓	✓	✓
Gastrointestinal disease/complaint	✓	✓	✓	✓
Acute diarrhea	✓	✓	< 2 months	< 6 months
Family history of CaCR or polyposis	—	✓	✓	✓
Travel to tropical regions in the last 3 months	Excluded if < 6 months	✓	✓	Not recommended
Health care worker	—	✓	—	✓
History of psychiatric disorders	—	—	✓	✓
Metabolic syndrome, malnutrition	Metabolic Syndrome was considered a relative criterion	—	✓	✓
Diabetes mellitus	—	—	✓	✓
History of malignancy or QT	✓	—	✓	✓
Immune disorders or use of immunosuppressants	Autoimmune disease was considered a relative criterion	—	✓	✓
History of neurological disorders or chronic painful syndrome	Relative criterion	—	✓	✓
Chronic use of PPI (≥ 3 months)	—	—	✓	✓
Angioedema, recent vaccine with live attenuated virus	—	—	✓	✓
Additional Considerations	Recent allergen intake to the receptor	Use of medicines with fecal excretion	Travel involving medical tourism	Known exposure to Malaria, Schistosomiasis, Chagas Disease and Tuberculosis

—, not mentioned; ✓, recommended; FMT, fecal microbiota transplantation; IAG, Instituto Alfa de Gastroenterologia; HC-UFMG, Hospital das Clínicas, Federal University of Minas Gerais; BMI, Body Mass Index; ATB, antibiotics; QT, chemotherapy, PPI, Proton Pump Inhibitor; CaCR, Colorectal cancer.

Table 5. Blood tests recommended for screening fecal donors

Exams	FMT workgroup recommendations (2011)	Amsterdam Protocol (2013)	International Consensus on Stool Banking (2019)	Protocol IAG HC-UFMG
HIV 1 and 2	✓	Combined antigen/antibody assay	✓*	HIV antibodies and p24 antigen
Hepatitis A virus	IgM	Total antibodies if IgM positive	✓*	IgG and IgM
Hepatitis B virus	HBsAg, anti-HBc (IgG and IgM), and anti-HBs	HBsAg and anti-HBs	✓*	HBsAg, anti-HBc (IgG and IgM), and anti-HBs
Hepatitis C virus	Anti-HCV	Anti-HCV	✓*	Anti-HCV (total antibodies)
Hepatitis E virus	—	—	✓*	—
HTLV 1 and 2	—	Antibodies	—	Antibodies
Cytomegalovirus	—	IgG and IgM	±	—
Epstein-Barr virus	—	IgG and IgM	±	—
Treponema pallidum	RPR and FTA-ABS	Haemagglutination test	✓*	VDRL
Trypanosoma cruzi	—	—	—	Antibodies (hemagglutination and indirect immunofluorescence)
Strongyloides stercoralis	—	ELISA	✓*	—
Entamoeba histolytica	—	Agglutination and *dipstick test*	—	—
Schistossoma mansoni	—	—	—	IgG
Blood Cell Count	—	—	✓	✓
Metabolic panel	—	—	✓	✓
Hepatic panel	—	—	✓	✓
C-reactive protein	—	✓	✓	✓

—, not mentioned; ✓, recommended; *, laboratory technique according to national or regional protocol; ±, can be considered; FMT, fecal microbiota transplantation; IAG, Instituto Alfa de Gastroenterologia; HC-UFMG, Hospital das Clínicas, Federal University of Minas Gerais; HIV, human immunodeficiency virus; HTLV, human T-lymphotropic virus; IgM, immunoglobulin M; IgG, immunoglobulin G; HBsAg, hepatitis B antigen "s"; anti-HBc, hepatitis B antigen "c"; RPR, rapid plasma reagent test; FTA-ABS, fluorescent treponemal antibody absorption test; HCV, hepatitis C virus; ELISA, enzyme-linked immunosorbent assay; VDRL, venereal disease research laboratory.

Table 6. Stool tests recommended for screening fecal donors

Exams	FMT workgroup recommendations (2011)	Amsterdam Protocol (2013)	International Consensus on Stool Banking (2019)	**Protocol IAG HC-UFMG**
C. difficile	PCR for Toxin B, EIA for Toxins A and B	Toxins (ELISA) and toxigenic culture	✓	GDH + toxigenic culture
Adenoviridae	—	—	✓	—
Norovirus	—	—	✓	PCR
Rotavirus	—	—	✓	PCR
Coronavirus	—	—	—	PCR
Escherichia coli O157	—	—	✓	Isolation and PCR
Culture of enteric pathogens (*Salmonella, Shigella, Campylobacter, Vibrio cholerae,* Yersinia*)*	✓	✓	✓	Culture isolation
Salmonella sp. (PCR)	—	—	—	Isolation and PCR
Clostridium perfringens (PCR)	—	—	—	Isolation and PCR
Campylobacter sp. (PCR)	—	—	—	PCR
Multidrug-resistant organisms (MRSA, VRE, ESBL, carbapenemase-producing enterobacteriaceae)	—	—	✓	Swab and culture
Microscopy for eggs and parasites (3 serial samples)	✓	✓	✓	Addition of Baermann-Moraes
Giardia lamblia	✓	—	✓	Antigen
Entamoeba histolytica	—	—	—	Antigen
Cryptosporidium spp	✓	—	✓	Microscopy
Isospora and Microsporidia	✓	—	✓	Microscopy
Helicobacter pylori	—	—	If upper gastrointestinal via	—

—, not mentioned; ✓, recommended; FMT, fecal microbiota transplantation; IAG, Instituto Alfa de Gastroenterologia; HC-UFMG, Hospital das Clínicas, Federal University of Minas Gerais; PCR, polymerase chain reaction; EIA, immunoenzymatic assay; ELISA, enzyme-linked immunosorbent assay; GDH, glutamate dehydrogenase; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; ESBL, expanded-spectrum beta-lactamase producer

MDRO have become a public health challenge and should be researched in fecal donation candidates [60]. The risk of acquiring MDRO is particularly high after traveling to India, Asia and North Africa [61]. However, also in Europe, the prevalence of multidrug-resistant intestinal bacteria can reach more than 50% [60].

The FDA issued a recent warning about screening for MDRO in fecal donors after the death by *Escherichia coli* ESBL invasive infection in a FMT receptor [62]. Previously, MDRO research was not recommended by the FDA, although some study centers have already made this assessment. After the publication of two serious invasive infections due to ESBL, one complicated by death, the FDA went on to recommend that screening donors should include questions about risk factors for colonization. Individuals at high risk of colonization should be excluded during clinical evaluation. Examples of high risk are: health professionals, people with a history of recent hospitalization or a stay in long-term health institutions, people who regularly attend medical clinics, or outpatient surgery centers [62].

Travel to tropical countries in the last three months is cited as an exclusion criterion in several studies [9,39,63]. The concern is justified by the occurrence of traveler's diarrhea and the possibility of becoming asymptomatic carrier of pathogens. Paramsothy *et al.* considered the following areas as high risk for traveler's diarrhea: Africa (except South Africa), Middle East, Asia (except Japan and Thailand), Pacific (except Australia and New Zealand), Central America and South America (except Argentina and Chile) [39]. Candidate who visited Brazil was automatically excluded from the screening process.

Currently, the destinations considered high risk for traveler's diarrhea are South and Southeast Asia, South and Central America, most African countries, Eastern Europe and some Caribbean islands [64]. The main etiologic agents involved in traveller's diarrhea are *E. coli* enterotoxigenic, *Campylobacter*, *Salmonella*, *Shigella* and norovirus [65]. Latin American travelers may also experience norovirus diarrhea, *Giardia*, *Cryptosporidium* and *Entamoeba* [65]. Therefore, in order to structure a transplant center in countries such as Brazil, it is necessary to include targeted research for such pathogens during donor screening. Although travel to tropical countries is considered an exclusion criterion by international protocols, the present study shows that it is possible to structure a safe screening program, even in emerging countries like Brazil.

Gastrointestinal infections caused by viruses, bacteria and parasites must also be excluded, especially in asymptomatic carriers. In addition, these infections can promote transient changes in the intestinal microbiota even after eliminating the pathogen, which motivates the contraindication of donations for up to six months [66]. Several studies use as an exclusion criterion patients with risky sexual behavior, sexually transmitted diseases, use of illicit drugs, and history of incarceration [15]. The use of antibiotics in the last three months also makes the donation unfeasible. Antibiotics promote significant changes in the microbiota and normalization uses to occur three months after the end of treatment [67].

The chronic use of a proton pump inhibitor (PPI) is considered an exclusion criterion for stool donation. Several studies describe an association between chronic use of PPI and *C. difficile* infection [68]. In addition, it is known that PPI are associated with an increased risk of small intestinal bacterial overgrowth, a condition that can impact the donor's intestinal microbiota [69]. However, a minority of studies exclude chronic users of PPI [52].

LABORATORY CRITERIA FOR DONOR SELECTION

All candidates who pass the medical interview must undergo blood and stool tests to assess possible conditions that may confer an increased risk of transmitting infections. The main purpose of laboratory tests is to identify asymptomatic carriers and/or detect prohibitive subclinical changes. Large studies on donor selection have shown that a high number of candidates are excluded because they are asymptomatic carriers of pathogens [9,39,63]. However, there is significant heterogeneity in the screening tests between the various studies on FMT. A systematic review selected 168 articles on screening of 1513 donors and found that it was incomplete in more than 50% of published studies [52].

The main pathogens investigated in clinical trials are: human immunodeficiency virus (HIV), hepatitis A, B and C virus, syphilis, *C. difficile*, *Salmonella* sp., *Shigella* sp., *Campylobacter* sp., *Cryptosporidium* sp. and enteric parasites. Less than half of studies researched Giardia, Yersinia, *E. coli* O157 and *H. pylori* and less than a third cytomegalovirus, human T lymphotropic virus and Epstein-Barr virus [52]. It is unanimous that donors need to be tested for human immunodeficiency virus (HIV),

hepatitis B and hepatitis C virus. It is also recommended to search for the Human T-Lymphotropic Virus (HTLV), multiplex PCR to detect rotavirus, norovirus and adenovirus according to local epidemiological specificities [47].

Common reasons for donor exclusion based on fecal examinations in developed countries were the detection of *Dientamoeba fragilis, Blastocystis hominis, C. difficile* and rotavirus. Exclusion by serological tests was less frequent. There are reports of exclusion due to apparent exposure to *Strongyloides* and indeterminate serum levels of antibodies against hepatitis C virus [9,39,63].

The search for Epstein-Barr virus (EBV) and cytomegalovirus (CMV) can be considered. There is no agreement on the selection or exclusion of these donors. The European Consensus on FMT recommends that individuals with positive EBV and CMV serology should be excluded [47]. On the other hand, the International Consensus on stool bank recommends that donors positive for CMV or EBV should not be excluded. As an option, only cases of active or recent infection (positive IgM) should be excluded [22]. The reason is that there is a high prevalence of previous exposure to both viruses and there are no reported cases of diseases associated with CMV or EBV attributable to fecal transplantation, even among immunosuppressed individuals. It is estimated that the seroprevalence of the Epstein-Barr virus in the world population is 90-95% [70]. The same trend is observed in relation to CMV, with seroprevalence ranging from 80 to 100% in emerging countries [71].

In Brazil, few studies on the prevalence of infection by EBV and CMV have been published. A study carried out in São Paulo state, with healthy blood donors, demonstrated positive EBV IgG antibody in 94.44% of the samples [70]. A nationwide study that evaluated 1045 Brazilian blood donor samples found a prevalence of CVM IgG antibody in 96.45% of the candidates [72]. In Minas Gerais state, IgG positivity for CMV in pregnant and postpartum women is greater than 85% [73]. In this scenario, the validity of EBV and CMV serology in healthy donors already submitted to a rigorous clinical questionnaire is debatable.

The presence of nematodes and protozoa infection should be assessed based on the clinical, social and geographical characteristics of each region. Special attention to *Strongyloides stercoralis* due to the risk of disseminated infection, especially in

immunosuppressed individuals. The isolated presence of symptoms should not be used for parasitic diseases such as strongyloidiasis, since most patients with chronic infection are asymptomatic. Based on parasitological examinations, the occurrence of strongyloidiasis in southeastern Brazil is 3.9% [74]. The most common method for diagnosis is direct microscopy of the parasite in the stool. However, during chronic infection, the detection of the pathogen may be intermittent, with sensitivity in a single sample of up to 66% [75]. To increase the sensitivity, it is recommended to repeat samples collection or concentration techniques such as Baermann-Morais method [76]. Serological tests can also be used, with sensitivity around 70-97% and specificity of 87-100% [77]. However, patients may experience false-positive reactions with other nematodes, especially in regions of higher prevalence, reducing the positive predictive value of the test. In addition, individuals may also have persistent positive antibodies after successful therapy, limiting the value of this test in the diagnosis of active infection.

Brazil is an endemic country for *Schistossoma mansoni* infection particularly found in states in the northeast region and some regions of Minas Gerais state. The gold standard for diagnosis is the oogram - fresh examination of material resulting from the collection of six to nine fragments of the Houston valves. However, the test requires an invasive procedure impracticable in healthy donation candidates. Serological tests are mainly directed against antigens of *S. mansoni*. They are highly sensitive, but moderately specific, and therefore a good tool for screening patients in endemic areas. Antibodies remain detectable for long periods after treatment and, consequently, serology does not differentiate between active and previous infection.

Giardia duodenalis is a protozoan with worldwide distribution, found especially in areas of poor sanitary condition [78]. It causes epidemic or sporadic diarrhea, with emphasis on groups considered to be at high risk such as infants, travelers and immunosuppressed. The most widely used diagnostic method is direct microscopic examination of stool. Microscopy is specific for the detection of trophozoites and Giardia cysts. Nevertheless, it has certain limitations such as reduced sensitivity in a single stool sample. To increase the sensitivity, it is necessary to perform a serial collection of three samples since the cysts are eliminated intermittently. For healthy donors without gastrointestinal symptoms, the test is aimed at researching cysts, with

the trophozoites being the forms most commonly found in liquid stools. On the other hand, methods for detecting antigens in feces show better diagnostic performance, with sensitivity of up to 82% and specificity of 91.5% in some studies [78].

Brazil is also considered a country with a high prevalence of amoebiasis [79]. Approximately 90% of infected individuals are asymptomatic carriers, which makes screening this pathogen essential in asymptomatic donors. The diagnosis of amoebiasis can be made with microscopy, serology or fecal antigens. Serological tests have a sensitivity of 90 to 93% [79], but are of little use for diagnosis in endemic areas since they are not able to distinguish acute infection from previous contact. A negative result in an asymptomatic donor is useful in excluding the disease and a good tool for screening. Microscopy is able to identify cysts or trophozoites in the stool. However, it is necessary to collect three samples on alternate days to achieve a detection rate of 85-95%. As a disadvantage, microscopic examination is not able to differentiate *Entamoena histolytica* (pathogenic form), from *E. dispar* or *E. moshkovskii* (non-pathogenic forms) [79]. The detection of fecal antigens is more sensitive than microscopy and is useful in this differentiation.

The search for *C. difficile* must be performed in all donors. However, the best screening method in an asymptomatic population has not been standardized. The main studies used molecular tests for toxigenic strain (PCR) associated or not with a second method such as immunoenzymatic assay, glutamate dehydrogenase or toxigenic culture [9,39,63,80]. The gold standard method for the detection of *C. difficile* is the toxigenic culture. However, the test is not widely available, it requires more time to release the result and complex logistics for its execution. Many laboratories have commercial assays for combined antigen/toxin detection and/or molecular assays for detecting the tcdA or tcdB gene. These are standardized tests for symptomatic patients with unformed stools. Previously, a commercial nucleic acid test demonstrated a higher negative predictive value for symptomatic patients than glutamate dehydrogenase or a multistage test algorithm [81]. Nonetheless, a recent detection study of *C. difficile* in asymptomatic patients shows that GDH has a negative predictive value of 99.3%, very similar to the commercial nucleic acid amplification test (NAAT) - 99.9% [82]. Such findings suggest that the use of GDH or NAAT, complemented or not with toxigenic culture, is

appropriate for screening asymptomatic donors, as is done in the FMTC of the IAG-HC/UFMG.

Some researchers recommend biochemical and hematological analysis such as complete blood count, C-reactive protein, renal function, hepatic and metabolic biochemistry to exclude relevant undiagnosed diseases. C-reactive protein, despite being a nonspecific inflammatory marker, is useful in identifying an underlying inflammatory state not assessed during clinical evaluation.

Other tests may be recommended. Fecal calprotectin has been used in some studies because it is a good screening test in patients with diarrhea [47]. However, its use in healthy and asymptomatic adults remains unknown, which justifies not using this method in the present study. Chronic infection with *Helicobacter pylori* should be investigated if the route of administration of the transplant through the upper gastrointestinal tract as a nasogastric catheter or by lyophilized capsules. Such research is not necessary if the FMT is by colonoscopy [47]. There is insufficient data to indicate screening for fungi in possible donors.

Little is known about the ideal donor screening criteria. Consequently, all possible candidates are subjected to extensive screening methods. Studies describe that the rigor in the selection is high, with eligibility rates that can reach 3%, which makes the recruitment of donors a limiting factor for FMT [22,83,84]. In other studies, only 10 and 32% of possible candidates met all the criteria for donation [39,63]. In the present study, criteria similar to those recommended by the main protocols were used (Tables 5 and 6), with donor detection rate similar to that described in the literature. The overall detection rate was 3% while the detection among potential donors was 12.1%.

SCREENING FREQUENCY IN DONORS

There is no consensus on the timing and frequency of examinations at loyal donors. Stool can be donated daily and repeating the complete blood and stool screening with each donation is unreasonable. The recommended frequency of exams varies from once every four weeks, every four months, and up to every six months [9,21,39,40]. Rode *et al.* recommend that donated feces remain in quarantine for 30 days until further clinical and laboratory screening (blood and stool tests) is carried out and the material is then

released for clinical use [85]. In OpenBiome, feces are collected for 60 days after the first screening and remain in quarantine until the second (60 days). A third screening is performed later for conditions with a possible seroconversion window [41]. The International Stool Bank Consensus recommends that donors undergo a full clinical and laboratory evaluation every 8 to 12 weeks [22]. It also recommends that the donated feces be subjected to rapid molecular testing for pathogens directly before the infusion or that they remain in quarantine until the donor has undergone a new additional screening at the end of the period and remains eligible [22]. The ideal frequency of screening for loyal donors has not been defined. Complete screening is recommended every 3 to 6 months or more frequently if the donor becomes symptomatic or if changes in risk factors occur [47].

CHALLENGES IN THE SCREENING AND SELECTION PROCESS

A challenge to maintaining a stool bank is the high rate of patients lost to follow-up due to the significant demand for time in conducting tests and donating stools. This trend was observed in the present study. Observational data from a large US stool bank shows a high dropout rate during the donor screening process. About 23.5% of 15317 candidates (3599 people) lost track during some stage of the process [86]. The real reasons were not explained, but the financial burden and time available are possible related factors. Some places admit financial compensation for the expenses and time demanded in the process. However, the financial compensation for tissue and cell donation is still discussed around the world. In Europe, for example, compensation for donation is not allowed. In the United States, funding is allowed for donation of certain materials such as plasma and sperm. In Brazil, there are no specific regulations on fecal donation. The ordinance of the Ministry of Health, which regulates the National Transplant System, signs that organ and tissue donors and their legal guardian(s) cannot receive any remuneration or any other type of material compensation or financial by the act of donation [87].

Finding donors and keeping them loyal in a fecal transplant program is a challenging task. A study carried out with the Australian transplant center showed that logistical difficulties, such as frequency of exams and a long follow-up period, represent an

impediment for about 40% of potential donors [39]. In addition, only 2-12% of potential candidates are able to complete all stages of the screening process and initiate donations [88]. To avoid shortages and keep the transplant center functioning, it is recommended that recruitment be done on a continuous and regular basis.

Out of six donors approved during the entire selection process, only two remained loyal for more than six months. Problems such as displacement for exams, difficulties at work and loss of follow-up were the main obstacles observed.

DEGREE OF RELATIONSHIP BETWEEN DONOR AND RECEIVER

Historically, the first transplants were performed with fecal samples from family members. In the impossibility of recruiting a related donor, receptors received material from unrelated volunteers [89]. With the advent of stool banks, an inversion of priority was observed, with an increasingly use of feces from universal voluntary donors [83].

Donors' relatives have the disadvantage of embarrassment during the family approach. Zipursky *et al*. demonstrated that almost a third of the receptors considered the conversation with family members to obtain feces unpleasant [90]. In addition, the experience of blood centers shows that the detection of infection in targeted blood donors (relatives or friends) is greater than donations from unrelated volunteers [91]. A possible explanation is that relatives and friends, in the desire to help, omit small complaints during the screening.

An advantage with the use of unrelated donors is the possibility of providing material quickly when the treatment is indicated. The process of finding a donor is time-consuming, requires a multi-person approach and significant expenditure on blood and stool tests. Performing the entire screening process among family donors may be impractical depending on the urgency of the transplant. The use of universal donors reduces time and costs related to various stages of selection.

Systematic reviews and meta-analyzes found no significant difference regarding the effectiveness and side effects of FMT when comparing donors related to unrelated volunteers [92-95]. Current evidence indicates that the success of FMT does not depend on the degree of kinship of donors. However, there is a preference in the use of

universal donors, unrelated, due to standardization, reproducibility, security in screening and better cost-effectiveness [47].

FRESH *VERSUS* FROZEN STOOLS

FMT can be performed by administering freshly collected or frozen fecal samples. To use fresh samples, it is necessary to articulate all logistics between feces collection, material preparation and infusion. It is recommended that the transfer of fresh feces takes place within the first 6 hours after evacuation, and should be stored in hermetically sealed containers at a temperature of 2 - 8 °C [15]. The difficulties faced in this process led to the use of frozen samples that would be previously prepared and would be readily available when necessary. The main studies using frozen fecal samples show a CDI resolution rate between 71 to 100% [46,96-102]. A similar rate is found when using fresh stools. Three randomized clinical trials compared the use of fresh and frozen samples and found no significant difference in their primary outcomes [96,99,100]. Contrarily, a lower resolution rate was reported in a pilot clinical trial using frozen feces in seven patients affected by CDI [101]. The rate was 71.4% and can be attributed to episodes of severe CDI, refusal to receive a second transplant and absence of intestinal lavage prior to the procedure [101].

AMOUNT OF FECAL SAMPLE

There is no consensus on the exact amount of fecal sample needed for transplantation. There is evidence of therapeutic success with 30 g and 200 g samples [97,103]. The minimum amount of feces with documented therapeutic success is 25 g for infusion in the lower gastrointestinal tract and 12.5 g for upper tract [104]. Nonetheless, patients transplanted by CDI obtained better results with the use of larger quantities [93]. A systematic review showed a higher risk of therapeutic failure (up to four times) with the use of infusions prepared with less than 50 g of fecal substrate compared to preparations with more than 50 g [93].

Regarding the diluent, solutions prepared with sterile saline have a lower recurrence rate, greater reproducibility and standardization during sample preparation [93]. Approximately 50 g (minimum 30 g) of fecal substrate is mixed and diluted to 150-250

mL of sterile 0.9% saline. The ideal volume is discussed, and preparations between 30 mL to 500 mL can be used, with an average of 250 mL [104,105]. However, there is a higher rate of CDI resolution with administration of larger volumes (97% with 500 mL, 86% with 200 – 500 mL and 80% with less than 200 mL) [93]. For infusion in the upper route, caution is recommended with the volume administered due to the risk of regurgitation and aspiration pneumonia with larger volumes [105].

SAMPLE PROCESSING AND STORAGE

To guarantee the viability of samples it is necessary that they are processed and stored within 6 to 8 hours [106]. The fecal microbiota remains stable for up to 8 hours when kept cooled to 4 °C [107]. After this period, there is a gradual reduction in microbial viability related to reduction of its diversity. Freezing in this phase should be avoided as it can disrupt the bacteria structure and affect the quality of samples [108].

The stool processing method differs according to the route of administration. For colonoscopy, whose working channel has a diameter ranging from 2.8 to 3.2 mm, it is necessary that the fecal sample be diluted, homogenized and filtered in order to remove dietary fibers or coarse dirt that may obstruct the canal of the device. After filtration, it is recommended to use cryoprotection with glycerol if the samples are stored under freezing. Cryopreservation is a fundamental step in the creation of a stool bank, as it does not compromise the clinical effect of FMT, prevents the crystallization of the material and allows treatment on demand [96].

The main diluent used is sterile 0.9% saline. Water, milk, yogurt and saline with *psyllium* were also used, but without evidence in the literature that favors one in particular [15]. Nevertheless, sterile saline is a standard diluent, less likely to interfere with the microbiota. The amount needed varies and depends on the consistency of the stool. In general, 200 mL of saline solution is used for each 50 g of stools. The final viscosity of the suspension must be as high as possible to allow adequate passage through the working channel of the colonoscope and remain in the intestine for the longest time [53].

It is not necessary to use an anaerobic chamber to process the samples. In most studies, manipulation occurs under aerobic conditions without impairing the effectiveness of the

treatment [46,96-102]. However, to avoid overgrowth of aerobic bacteria, the preparation should be as short as possible and the samples should be conducted under refrigeration at 2 - 8 °C until final freezing at -80 °C [53].

FREEZING TIME AND SPECIMEN VIABILITY

Another important aspect to be considered with frozen samples is the viability of the microbiota over the storage time. Studies show that storage conditions of fecal samples affect the microbiota composition, although major changes occur after storage at room temperature for more than 24h [109,110]. Costello *et al*. evaluated the viability of the microbiota of fecal samples frozen at -80 °C for six months of storage and demonstrated that it remains practically unchanged during this period [111]. CDI resolution can be achieved with storage time of 6 months, 10 months and up to one year, with frozen preparations containing glycerol as a cryoprotectant at -80 °C [5,46,102,111]. Similar results have been reported in animal model studies although a decline in microbiota diversity is observed after seven months of storage [112].

Storing fecal suspensions at -20 °C for up to 30 days is also effective in FMT for recurrent CDI. However, at this temperature, the proportion of Firmicutes/Bacteroidetes was significantly higher in fecal samples frozen for more than 50 days compared to fresh samples from the same donor [113]. Fecal suspensions can be safely maintained at -20 °C for up to two months without compromising the effectiveness of treatment [108].

It is recommended that fecal suspensions be stored for up to two years and can be used for transplantation within 1 year after donation if they have been stored at -80 °C [22]. After two years the stored material must not be used. It must be disposed of according to the local medical waste management procedure. However, a small aliquot of stool from each donor must be stored to ensure traceability in the event of future adverse events.

FMT INDICATION: RECURRENT AND REFRACTORY *C. DIFFICILE* INFECTION

FMT is recommended for the treatment of recurrent CDI, regardless of severity. Patients with at least two previous episodes of CDI undergoing standard antimicrobial treatment and with no sustained cure should be considered for FMT [47]. The recommendation is based on the high rate of symptom resolution achieved with the restoration of a healthy microbiota. In addition to efficacy, FMT has a favorable safety profile that must be considered in the risk-benefit ratio for patients with recurrent CDI. Several studies have shown an excellent safety profile, especially in short-term follow-up, with reports of mild, self-limited and short-term adverse events, such as abdominal discomfort and flatulence [114].

Transplantation can also be indicated as a therapeutic option for refractory CDI. The resolution rate found in observational studies is approximately 55% [115]. Refractory cases appear to have a lower rate of response to FMT. British cohort with 124 patients showed a resolution rate of 91.0% for recurrent CDI and 73.0% for refractory CDI on the seventh day ($p = 0.007$). However, at the end of the third month of follow-up, the rates became equivalent (75.0% vs. 82.0%, $p = 0.4$) [116]. In addition, serious and complicated CDI can manifest as refractory infection, with persistence of symptoms despite antimicrobial treatment. In this context, FMT should be considered as an alternative to surgical treatment, especially in cases of severe CDI with early failure to antibiotics [12]. FMT should be weighted inclusive in severe-complicated and refractory CDI to the first antimicrobial treatment [47]. Mortality from severe and complicated CDI, requiring a colectomy, can reach 80%, especially in cases where surgery is performed late [10]. The cure rate for FMT in this scenario is 66-88% [117]. The colonoscopic route was the most used in these cases and some authors maintained the use of vancomycin after the procedure [117]. Regarding fulminant CDI, defined as severe CDI accompanied by arterial hypotension or shock, toxic ileus or megacolon that did not respond to the optimized clinical treatment, the FMT being an option to be considered in the subgroup of patients with high surgical risk [22].

An important issue still to be listed is the possibility of transferring a microbiota with harmful characteristics that will manifest after decades [12]. Thus, it is recommended that FMT be indicated in clinical practice only for the treatment of conditions in which there is a high level of evidence. Conditions such as inflammatory bowel disease, irritable bowel syndrome, metabolic disorders, hepatobiliary disorders, autism appear as possible

clinical indications for FMT, but still with no strong evidence-based recommendation [12]. There is also insufficient evidence to recommend FMT as a treatment for the first episode of CDI [12].

PRE-TRANSPLANT ANTIBIOTICS MANAGEMENT

Patients with recurrent CDI should be treated with antibiotics for at least 3 days before FMT in order to reduce the load of *C. difficile* and improve the result with the transplant [12]. Vancomycin has been used at a dose of 125 – 500 mg, orally, four times a day for three, five, ten or more days before the procedure [9,18,96]. Antimicrobial therapy should be stopped 12-48 hours before infusion of fecal substrate to avoid negative effects on the transplanted microbiota. In emergency cases, when frozen samples are immediately available for transplantation, bridge therapy with antibiotics can be dispensed with [12].

BOWEL PREPARATION

It is not yet clear whether intestinal cleansing is really necessary for the success of FMT. However, even with a low level of evidence, the European Consensus on FMT recommend intestinal preparation with polyethylene glycol (also known as PEG or macrogol) before the procedure, even if the FMT is performed through the upper gastrointestinal or colonoscopic route [11,12]. The rationale for this measure is the load reduction capacity of *C. difficile* after intestinal washing [9,118].

Van Nood *et al*., in a randomized clinical trial, compared three treatment regimens for recurrent CDI: (1) isolated use of vancomycin, (2) vancomycin associated with intestinal cleansing in fourth or fifth day of treatment and (3) FMT by feces infusion via nasoeduodenal tube preceded by a short period of vancomycin (4-5 days) and intestinal cleaning with four liters of macrogol solution [9]. Thirteen of the 16 patients (81%) achieved CDI resolution after the first FMT and two of the remaining three after a second infusion, resulting in an overall success rate of 94%. The resolution of CDI in the other two groups was significantly lower. In patients treated with vancomycin alone, four out of 13 achieved CDI resolution (31%). In the group that received vancomycin with intestinal cleansing, only three of the 13 patients (23%). Intestinal cleansing, as an

isolated intervention, was not effective in resolving CDI. On the other hand, associated with FMT, it caused a cure rate of up to 94%.

Lee *et al*., in a randomized clinical trial, evaluated FMT via enema using fresh frozen feces to treat recurrent CDI without colonic preparation [96]. Similar to other studies, patients received suppressive treatment with antibiotics that were interrupted 24 to 48 hours before transplantation. Even without intestinal cleansing, the success rate achieved was 83.5% with frozen stools and 85.1% with fresh stools.

Fischer *et al*., in a retrospective multicenter study, evaluated the effectiveness of FMT according to intestinal preparation. Among the 413 patients undergoing FMT via colonoscopy, the quality of preparation was classified as excellent, regular and poor in 67%, 22% and 11% respectively [119]. Among those who did not respond to FMT, 15% had adequate preparation, 24% regular and 35% poor ($p = 0.003$, univariate analysis). However, after including other risk factors for therapeutic failure in the analysis (severity of CDI, hospitalization and previous number of CDI), the variable intestinal cleansing did not persist in the final model of the multivariate analysis. Compared to the group of patients with adequate bowel preparation, the *odds ratio* for FMT failure was 1.16 (95% CI: 0.57-2.37; $p = 0.68$) for regular preparation and 1.64 (95% CI: 0.69-3.88; $p = 0.26$) for poor.

Intestinal cleaning is safe and allows an adequate study of the ileocolonic mucosa. However, it is capable of promoting changes in the microbiota with a substantial reduction in microbial load by up to 31 times [118]. It also considerably alters its composition, with loss of individual microbial specificity in up to 22% of patients undergoing intestinal preparation with polyethylene glycol [118]. Yet, 14 days after bowel preparation, the microbiota of these individuals recover, resembling their original form [118].

There are several purgatives that can be used in intestinal cleansing. Polyethylene glycols are non-absorbable isosmotic solutions, with an excellent safety profile, which pass through intestine without absorption or liquid secretion, with minimal impact on the volume or electrolyte composition of patients. The preparations must be diluted in large volumes of water (up to 4 liters) to obtain the desired cathartic effect. Compliance is best with split dose regimens, with 2 liters the day before the exam (usually at night)

and 2 liters the next morning (specifically for exams in the afternoon). Due to its safety profile, it can be used even in patients with chronic kidney disease, anuric, on hemodialysis [120].

ROTE OF ADMINISTRATION

FMT can be performed by inserting a nasoenteric, nasogastric tube, via gastroduodenoscope, enteroscope, by capsules containing lyophilized material, by colonoscopy, sigmoidoscopy or retention enemas. There is no method that is proven to be more effective than another. The choice depends on the particularities of each patient and the logistics of the transplant center. A study that compared the efficacy of the upper to the lower route found no significant difference between them. The success rate using the upper route was 88% (95% CI: 0.82-0.94) compared to 95% (95% CI: 0.92-0.97), using the lower (p = 0.162) [121]. All routes are effective for the treatment of recurrent CDI with numerical advantage, but not statistically significant, in relation to colonoscopy [92,122]. A recent systematic review showed that the majority of adult underwent FMT via colonoscopy (42%) followed by gastric or duodenal application (22%), enema (12%) or a combination of two methods (11%) [15].

Colonoscopy is considered by many to be the gold standard [98,123]. It has the advantage of the ability to visualize the colon and the possibility of infusing the material in the most affected intestinal segments, especially in the proximal colon, where the involvement by pseudomembranes is usually more severe [124]. Besides that, it is more physiological, allows the administration of a greater amount of feces, which would be related to the higher success rate found in some studies [125]. However, it has the drawback of intestinal lavage and the risk of perforation inherent in the procedure.

Fecal enemas are less invasive, easy to perform, cheaper and can be performed in an outpatient setting [126]. As a disadvantage, there is a shorter stool retention time, particularly in patients with sphincter hypotonia, a lower reach of the substrate in the colon (up to splenic flexure) and the need for multiple procedures to obtain efficacy [17].

The upper routes are fast and less expensive when compared to colonoscopy. Notwithstanding, they are aesthetically unpleasant and uncomfortable, especially the nasoenteric tubes where it is possible to monitor the infusion of fecal material through

the tube. To avoid regurgitation, a smaller volume is used, which can compromise the final result of the transplant [125]. Another concern is the degradation of the microbiota due to gastric acidity and the possibility of serious complications such as bronchoaspiration, hemorrhage and gastrointestinal perforation [127]. Wang *et al.*, in a systematic review, demonstrated that the rate of adverse events with upper route administration was higher in relation to the lower (43.6% and 17.17% respectively) [114]. The most serious side effects associated with FMT were reported via upper gastrointestinal tract, due to the risk of vomiting and aspiration. Three deaths related to FMT occurred after application to the upper tract, two cases of aspiration pneumonia and one case of septic shock secondary to toxic megacolon [128-130]. Cases of non-lethal aspiration pneumonia after vomiting of fecal suspension and a small intestinal abscess with nasojejunal route have also been reported [131].

There is also the possibility of FMT by enteroscopy. Ganc *et al.*, in 2015, published a successful Brazilian experience of 12 patients who underwent FMT by endoscopic infusion in the proximal jejunum. The resolution rate was similar to that described by other routes [36]. The endoscopic route, whether superior or inferior, should be performed only by trained endoscopists to reduce the risk of complications [22]. There is no data available on the FMT learning curve, but the opinion of experts is that professionals should perform at least ten transplants before being considered trained [22]. Capsules appear as a promising option for dispensing intestinal preparation and hospitalization. They are more aesthetically pleasing, less invasive and eliminate the risk of endoscopic perforation. However, they need technology for lyophilization and preparation of capsules resistant to gastric acidity. The lyophilization process is expensive and consists of production of powder substrate from the vacuum drying of fecal suspensions [132]. The rate of CDI resolution with capsules is similar to rates through the use of colonoscopy. Conversely, it presents as a limiting factor, the need to ingest a high number of capsules throughout the day (minimum of 30 capsules per day in some studies) [133]. In the short term, new technologies will most probably be incorporated and this procedure could occupy a prominent place.

POST-PROCEDURE CARE

Patients undergoing colonic FMT can receive loperamide, usually 2-4mg, to reduce intestinal transit time and improve colonization conditions of the microbiota [53]. It is also recommended that patients remain in the right lateral decubitus position or in Trendelenburg to increase the retention time of the transplanted fecal material [53]. But there are few studies that describe this recommendation, based only on expert opinion. There are no studies proving the effectiveness of its use.

NUMBER OF TRANSPLANTS

A second FMT may be required in case of failure or recurrence after the first attempt [134]. In severe CDI, especially in colitis with endoscopic evidence of pseudomembranes, repeated fecal transplants may be necessary [12]. Among outpatients with recurrent CDI, a single colonoscopy infusion has a cure rate of 85 to 91% while patients with severe or complicated CDI can achieve an equally high cure rate from repeated courses of antibiotics and fecal infusion [135]. Most patients achieve CDI cure with one or two FMT combined with vancomycin and only a minority require three or more [135].

Cammarota *et al.* successfully treated patients with pseudomembranous colitis from colonoscopy infusions every 3 days [18]. However, to achieve a high success rate, an average of two to three procedures per patient was required.

Fischer *et al.* demonstrated that leukocytosis (count above 22.6 x 10^3 cells/mm^3), hypoalbuminemia (serum albumin below 2.3mg/dL), presence of pseudomembranes at the first colonoscopy and use of antibiotics for other infections (non-CDI) are predictive factors for new transplants. The presence of pseudomembranes and the use of other antibiotics increase the chance of a new procedure by six and three times respectively. In the study, 47.4% of patients required two or more transplants. Only one required five infusions [135].

TRACEABILITY

The entire process involved in the FMT must be carried out and monitored with strict quality control. From the inclusion of donors to the administration of fecal substrate it is

necessary to take measures to ensure a high standard of quality and complete traceability. Measures include education of the personnel involved, validation of laboratory procedures and equipment, and recording of information. Donor data, laboratory tests, fecal samples should be stored for up to 10 years to ensure traceability. It is advisable to store fecal samples from receptors before and after transplantation, as well as donors to allow follow-up in case of future adverse effects [47].

ADVERSE EVENTS AND SAFETY

FMT is considered a safe, well-tolerated therapeutic method with few adverse events (AE), generally self-limited and short-termed [136]. Most clinical trials and systematic reviews show that AE related to FMT are minor events, observed transiently after procedure and with short-term spontaneous resolution. The main ones are diarrhea, abdominal discomfort, constipation and fever. Severe events are uncommon and are often associated with complications related to sedation or endoscopic procedure [9,11,16,99,127].

Wang *et al.*, in a systematic review with 1089 patients, found occurrence of AE in 28.5% of fecal transplants [114]. There was a higher rate of AE in patients undergoing FMT through the upper gastrointestinal tract (43.6%) compared to the lower tract (17.7%). However, the occurrence of severe events was 2.0% in high route and 6.1% in low route. The main ones related to the upper way were abdominal pain, nasal congestion, sore throat, rhinorrhea and gastrointestinal bleeding. Abdominal discomfort followed by transient fever was the most common event in both routes. Patients with inflammatory bowel disease had more fever than those with CDI (7.9% vs 2.0%, p = 0.011). A total of 44 severe AE occurred in 9.2% of the patients being death (3.5%, 38/1089), infection (2.5%, 27/1089) and reactivation of inflammatory bowel disease (0.6%, 7/1089). However, of the 38 deaths, only one was definitively related to FMT (bronchoaspiration during colonoscopy sedation) and two possibly related (pneumonia and peritonitis three days after FMT by nasogastric tube). Regarding the incidence of severe infection, eight cases were probably or possibly related and 19 unrelated. Of the eight infections, two were viral (cytomegalovirus and norovirus), two bacterial

(*Escherichia coli, Proteus mirabilis, Citrobacter koseri,* and *Enterococcus faecium*) and four without identified pathogen [114].

Lai *et al*., in an analysis of 5958 patients submitted to FMT since 2014, found a general incidence of adverse events of less than 1% [52]. The most common AEs were diarrhea (13.0%), distension/flatulence (11.6%), nausea/vomiting (6.1%), abdominal pain (5.5%) constipation (2.1%), fever (2.7%), headache (1.5%) and fatigue (1.4%). Severe AE were: aspiration pneumonia (0.16%), death (0.13%), sepsis (0.07%), intestinal perforation (0.07%), hospitalization (0.02%) and sedation-related complications (0.02%) [52].

FMT was shown to be safe in an immunosuppressed population with recurrent CDI. Shogbesan *et al*., in systematic review with 303 patients, most due to the use of immunosuppressive drugs, presented severe AE rate similar to immunocompetent patients. Nineteen patients (0.06%) had serious adverse (2 deaths, 2 cholectomies, 5 infections, 10 hospitalizations). Twenty-eight patients (9.24%) had minor AE such as abdominal pain, irritable bowel syndrome, nausea, and transient fever. There was no higher occurrence of infectious adverse events [136]. A clinical study on FMT in patients with moderate to severe ulcerative colitis, with follow-up time of up to 5 years, showed the occurrence of transplantation-related AE in 17.5% of cases, most of which were minor and short-term events. Most participants were on immunosuppressive therapy and received FMT by upper rout. Among the 57 reported AE, the main ones were fever, increased evacuatory frequency and abdominal pain during the first six hours [137].

In March 2020, the FDA issued a warning about the risk of severe infection with the use of FMT after notification of six patients who received fecal substrate from the U.S. feces bank for CDI treatment. Two patients developed infection caused by enteropathogenic *Escherichia coli* (EPEC) and four by shiga toxin-producing *Escherichia coli* (STEC). Four of the six patients required hospitalization. Two patients who developed EPEC infection received fecal substrate from two different donors. Four patients who developed STEC infection received the product from a single donor [138]. Previously, Azimirad *et al.* described two cases of immunosuppressed patients submitted to FMT because of CDI and who developed infection by *Clostridium perfringens* enterotoxigenic approximately 2 months after procedures [139]. This finding

reinforces the need to include the *C. perfringens* enterotoxigenic during donor screening.

In 2019, the FDA issued a warning about the risk of transmission of multidrug-resistant organisms. Two immunosuppressed adults submitted to FMT developed severe infection by *E. coli* ESBL, and one died. The material used in both procedures was obtained from the same donor, not tested for the presence of such bacteria. Until then, the research of MDRO had not been routinely performed in transplant centers. After the incident, the FDA issued a recommendation to investigate in donors risk factors related to colonization and direct research of MRSA, VRE, enterobacteriaceae ESBL and carbapenem-resistant Enterobacteriaceae [(140)].

After the warning from the FDA, the news of the death gained great repercussion in the media and scientific community. However, before the event, at least four deaths had already been described definitively or probably related to transplantation for recurrent CDI. First in an 88-year-old patient, with chronic obstructive pulmonary disease and atherosclerosis, who died 14 days after transplantation via nasogastric tube due to aspiration pneumonia [(130)]. Second death in an immunosuppressed patient, after solid organ transplantation, with cachexia due to advanced esophageal neoplasia, who died one day after FMT via colonoscopic due to bronchoaspiration during sedation [(141)]. Third patient, 68 years old, diabetic and with advanced oropharyngeal neoplasia, feeding by nasogastric tube, received transplant via tube and evolved on the third day with toxic megacolon, septic shock and death [(131)]. Fourth patient, 80 years old, previous history of vasculopathy, osteoarthritis and gout, evolved with septic shock by *E. coli* secondary to aspiration pneumonia and death after FMT via enteroscopic and under general anesthesia [(142)].

Patients submitted to FMT should be monitored for adverse events that can be attributed to the procedures. All AE potentially related to FMT must be registered. In addition to infections, the possibility of transmission of a phenotype associated with microbiota should be evaluated in the long term. Even though it is considered a safe method, the risks and therapeutic options should be discussed with the recipient before the procedure. A long-term study on FMT safety (10-year period) is currently underway, which will provide further clarification on the potential risk of developing such conditions [(143)].

COST-EFFECTIVENESS

In Brazil there are no epidemiological data on operating costs related to recurrent CDI. Nor comparative studies on cost-effectiveness of FMT. Based on international studies, it is known that CDI generates great burden on the health system with annual expenditures estimated at about $ 6.3 billion in the United States and cost per episode of CDI ranging from €5798 to €11202 in Europe [144,145]. An American cost-effective analysis compared three modalities of FMT (by colonoscopy, duodenal infusion and enema) *versus* standard antibiotic therapy (metronidazole, vancomycin and fidaxomycin) for the treatment of recurrent CDI [146]. The initial treatment of recurrent CDI with FMT via colonoscopy was the most economical strategy, with a cost-effectiveness rate of US$17,016 in relation to oral vancomycin. The same trend was observed when compared to metronidazole and fidaxomicin. It also concluded that, in places where FMT is not available, the strategy with oral vancomycin is preferable.

MICROBIOTA AFTER TRANSPLANTATION

Chang *et al.* demonstrated that after first episode of CDI the intestinal microbiota is little altered, remaining with predominance of dominant phylums Firmicutes and Bacteroidetes. Nonetheless, after recurrent episodes of CDI, there is a marked reduction in Bacteroidetes accompanied by a marked increase in other phylums that are usually a minority in intestinal microbiota [147]. Fecal transplantation reestablishes the initial microbial composition by promoting sustained alteration of the receptor microbiota, with a significant increase in the Firmicutes and Bacteroidetes and reduction of Proteobacteria and Actinobacteria [148].

Khoruts *et al.* compared the microbiota of a patient with recurrent CDI before and post-FMT. Prior to transplantation, the microbiota presented reduction of Bacteroidetes and increase in atypical bacterial populations such as *Veillonella*, *Clostridium*, *Lactobacillus*, *Streptococcus*. Two weeks after the infusion of fecal suspension, bacterial composition changed, becoming similar to that of the donor, characteristically marked by diversity, with predominance of Bacteroidetes [149].

Li *et al.* evaluated the composition of fecal microbiota after FMT in patients with metabolic syndrome undergoing autologous and allogeneic transplantation. They observed that the donor strains persisted for a period of three months replacing or coexisting alongside recipient strains. Colonization success was higher for species common to the donor and receptor than in relation to the new species inserted. In addition, receptors from the same donor exhibited varying degrees of microbiota transfer, indicating individual patterns of colonization and donor-recipient compatibility [(150)]. Another study on microbiota recovery in recurrent CDI showed that FMT significantly alters the microbiota in the long term and that the phylogenetic profile of the recipient is similar to that of the donor for up to one year [(136)].

PATIENT'S VIEW OF FECAL TRANSPLANTATION

FMT faces cultural issues that may hinder its acceptance. It is speculated that there is a low receptivity to treatment, justified in part, by its unpleasant nature. Nevertheless, studies on patient acceptance show that up to 94% of them would choose to receive FMT if it was required [(90)].

Patients with recurrent CDI experienced prolonged suffering with a debilitating disease, multiple hospitalizations and use of poorly palatable oral medications. Although culturally unpleasant, patients make a favorable judgment between recurrence risk and potential risks/benefits of FMT. Family support and educational level are also considered significant predictors of acceptance, particularly among married people, with children and those with higher education [(151)].

Even so, a small proportion of patients refuse treatment. One of the barriers is the "*yuck factor"* described as negative instinctive reaction in relation to a treatment considered dirty or unpleasant. However, the main factor of refusal is concern with the safety, especially the fear of disease transmission. Another factor is aversion to certain routes of administration, especially those using nasoenteric probes and the oral route. There is preference for the administration of fecal substrate directly in the colon, through colonoscopy. But the idea of ingesting odorless capsule is also well accepted [(5,90)].

Interestingly, physicians who treat patients with recurrent CDI can also act as a barrier to the FMT indication, either due to lack of knowledge, limited experience, safety

concerns, institutional and logistical barriers or concern about patient receptivity. An American study on the attitude of physicians towards FMT shows that almost a third of them did not indicate treatment in recurrent CDI because they believed that there would be a refusal by patients [90]. What is observed, however, is that there is a clear disagreement between the beliefs of physicians and their patients. The health professional plays an important role in the clarification and education of their patients. The involvement of both parties in the process of choosing a treatment is decisive for its acceptance.

CONCLUSIONS

Fecal microbiota transplantation has been considered a standard treatment for patients with recurrent or refractory *C. difficile* infection. Studies indicate that FMT is an effective therapeutic option, with a favorable risk-benefit ratio, in patients with failure to conventional antimicrobial treatment. Although transplantation appears to be safe, with few adverse effects, there is a theoretical risk of transmission of dysbiosis-related phenotypes. Long-term security data are needed to guide donor selection and rationalize interventions in the microbiota. In addition, many concepts on dysbiosis and microbiota manipulation are still under construction and their better understanding will provide subsidies for the use of a more effective and personalized treatment.

Recent identification of hypervirulent ribotype 027 and strains producing binary toxin in Brazil raises the warning about the need to optimize methods to face *C. difficile* infection. Incentives in health policies are necessary to expand the diagnostic and therapeutic capacity of a condition responsible for major epidemics in recent decades. The creation of a platform like the one presented, capable of providing treatment to serious and recurrent cases, is fundamental in addressing a growing condition in the country.

Fecal microbiota transplantation is a treatment modality under investigation. Thus, it needs scientific and ethical support for its application in daily clinical practice. It should be carried out in a research environment, in centers with experience in the CDI treatment and with the approval of local ethics committee, especially for conditions

beyond of *C. difficile* infection, whose evidence on benefit is still scarce. Its use outside these molds should be discouraged in Brazil.

The structuring of a Fecal Microbiota Transplant Center with a frozen stool bank allowed access to an innovative treatment modality that is not widely available in the country. Transplant centers with a stool bank allow to perform treatment on demand, less personalized, with more security and traceability. In addition, it allows standardization in the selection and manipulation of fecal samples, better evaluation of interventions, comparison of results and facilitates scientific communication.

Donor selection is a vital step in structuring a transplant center. However, finding healthy donors and keeping them loyal is a challenging task. This selection protocol used broad clinical criteria and was able to identify a large number of clinical contraindications prior to blood and stool tests. Our rigorous evaluation allowed us to identify contraindications in potential donors and rationalize the use of resources.

This article, as far as we know, was the first study to describe the experience in implementing a unit on fecal microbiota transplantation in Brazil. He sought to describe detailed instructions for structuring a fecal transplant center, such as regulatory and ethical aspects, selection of donors, processing and storage of samples, route of administration and post-procedure follow-up. The implantation steps described here should facilitate the safe dissemination of fecal transplant centers in emerging countries.

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8. ARTIGO 2

Experience of the first Brazilian stool bank in treating recurrent C*lostridioides difficile* infection with fecal microbiota transplantation

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ABSTRACT

Background: *Clostridioides difficile* infection (CDI) is the major cause of nosocomial diarrhea related to use of antibiotics in Brazil. The treatment of recurrent CDI is a challenge in countries where fecal microbiota transplantation (FMT) is not widely available. In addition, data on effectiveness and safety of FMT in emerging countries are scarce.

Objective: The main objective of this study was to describe the initial experience with fecal microbiota transplantation in the treatment of recurrent CDI in Brazilian cohort patients and to evaluate its effectiveness using frozen samples from stool bank.

Methods: In a prospective pilot study, FMT was performed using frozen samples from our stool bank. Donors were screened according to international guidelines and national regulatory aspects. FMT success was defined as cessation of diarrhea within eight weeks.

Results: Over two years, ten patients with recurrent CDI underwent FMT by colonoscopy using frozen samples. Median age was 68 (23 - 87) years, and 70% were women. Majority had Charlson comorbidity rate of 3 (0 - 6), severe CDI (54.5%) and a median of three previous episodes (1-4). In all patients, the diagnosis was confirmed with toxigenic culture. Average sample storage time was 39 (1 - 147) days. Overall resolution of CDI was 90%. Failure occurred in two procedures and were not related to severity of CDI ($p = 0.273$), bowel preparation ($p = 0.345$), comorbidities ($p = 0.809$), number of previous episodes ($p = 0.457$), or donors ($p = 0.164$). No serious adverse events were described during an average follow-up of 432 (36 - 782) days. Mild adverse events occurred in 54.5% of cases, mostly abdominal discomfort on the first day after the procedure. The two immunosuppressed patients did not experience any infectious adverse events.

Conclusion: FMT is a safe and effective treatment for recurrent CDI in small cohort of Brazilian patients. The implantation of a stool bank allowed to apply properly all the requirements needed to perform FMT in the country.

Key words: Fecal microbiota transplantation, *Clostridioides difficile* infection, stool bank.

INTRODUCTION

The human gut microbiota corresponds to the complex community of microorganisms that inhabit gastrointestinal tract and exert marked influences on the health of its host. It consists of bacteria, viruses, archaea, protozoa and fungi that live synergistically to maintain local homeostasis. It participates in vital functions, such as digestion, vitamin production, immune system development, and defense against external pathogens [1,2]. Throughout life, it is influenced by several environmental factors, such as lifestyle, geographic location, diet, and medication use, mainly antimicrobials.

When a breakdown of local balance occurs, the resulting microbiota has its composition altered and diversity reduced [3]. This process of dysfunction and microbiota imbalance is called dysbiosis. Dysbiosis has been associated with the pathogenesis of intestinal and extra-intestinal disorders, such as peripheral insulin resistance, obesity, neurological disorders, irritable bowel syndrome, inflammatory bowel disease (IBD) [4-7]. However, the greatest causal relationship between dysbiosis and illness is seen in recurrent *Clostridioides difficile* infection (CDI) [8].

C. difficile infection usually occurs in patients with antibiotic-induced dysbiosis. It usually affects hospitalized elderly people, with poor immune responses and who present microbiota imbalance due to recent use of antimicrobials. The first CDI episode is treated with metronidazole and / or vancomycin, with a success rate of around 80% [9]. Recurrence can be treated with fidaxomycin or vancomycin taper and pulse regimen for six to eight weeks [9]. However, the success rate with antibiotics decreases progressively as new recurrences occur. In patients with multiple relapses, 60% have a new recurrence if the antibiotic therapy strategy is maintained [10]. This finding can be explained by the persistence of dysbiosis cycle perpetuated by antibiotics associated with non-recovery of the microbiota.

The treatment of recurrent CDI is a challenge, especially in Brazil where fidaxomycin is not available. Repeating antibiotic doses is ineffective as it perpetuates the dysbiosis cycle. In this sense, using treatment capable of correcting dysbiosis and reestablishing a healthy microbiota is a fundamental step in the patient's recovery. Among the

possibilities of treatment, fecal microbiota transplantation (FMT) appears as an important option.

Fecal microbiota transplantation involves the transfer, through the gastrointestinal tract, of healthy microbiota, in order to repopulate the digestive tract and correct dysbiosis. It could reshape the intestinal microbiota, restoring their protective function against *C. difficile* and achieving therapeutic effects [11]. The material introduced is composed of all species diversity and metabolites present in the donor's feces, capable of exercising its functions for a prolonged time [12]. Unlike traditional antimicrobial treatment, FMT is highly effective in treating recurrent CDI with an overall cure rate of 90% [13-17]. It has few side effects, mainly mild and transient, being well accepted by patients and capable of improving their quality of life [18]. Although FMT still remains an experimental treatment, it is now recognized as CDI treatment option and is therefore recommended by medical societies for multiple recurrent CDI in patients who have failed to standard therapies [9,19].

FMT can be performed by infusing fecal material via upper or lower gastrointestinal tract. Techniques for administration can be by nasogastric / nasojejunal tube, gastroscopy, oral capsules, enema, sigmoidoscopy or colonoscopy [20,21]. The procedure is considered safe and, in most cases, free of serious adverse events, although some transient peri-procedure gastrointestinal symptoms may develop in some patients [22].

CDI is the most common cause of nosocomial infectious diarrhea and is associated with significant morbidity and mortality [23]. Its incidence, severity and recurrence have increased worldwide in recent decades [23]. However, it is more difficult to estimate the incidence of CDI in developing countries. Systematic review and meta-analysis found that the incidence of CDI in developing countries is 8.5 per 10,000 patient-days, comparable to that observed in the USA [24]. The CDI incidence in Latin America is likely to be underestimated due limited vigilance and awareness of this problem, and limited availability of diagnostic tools. Despite the underreporting and the few national epidemiological data, there is a growing record of *C. difficile* isolation and characterization in our country. In Brazil, CDI is recognized as the main cause of nosocomial diarrhea related to the use of antibiotics [25]. Recent study suggests that the CDI rate in Brazil is high despite the lack of epidemiological monitoring. The study found a point prevalence of 3.0 per 1,000 patient-days (95% CI, 1.9–4.8) of CDI in

hospitalized patients with diarrhea. Previous exposure to fluoroquinolones is a significant risk factor for developing infection [26].

In 2018, the hypervirulent *C. difficile* ribotype 027 (NAP1 / 027) strain was isolated for the first time in Brazil [27]. The strain has been responsible for increase in CDI cases since 2000, with outbreaks in North America, Europe, and Asia [25]. The ribotype had already been isolated in Latin America, but not yet in Brazil. However, despite the absence of national outbreaks to date, the identification of strains producing binary toxin and a new hypervirulant ribotype 027 in our country warns of the need to improve awareness, disseminate diagnostic tests and facilitate access to therapeutic measures, in particular TMF [27,28].

Despite the advent of recurrent CDI cases, FMT is not yet a reality in national clinical practice. There are few reports of fecal transplantation in Brazil. So far, only one study has been published in 2015, describing the experience of a small cohort of patients with recurrent CDI undergoing transplantation [29].

In Brazil, as well as in several countries in the world, there are no specific regulations for FMT. The current recommendation is that FMT be performed in reference centers for CDI, especially in hospitals with expertize and adequate logistics [19,30]. The International Consensus on Fecal Microbiota Transplantation also recommends that, in the absence of local guidelines, transplantation should be carried out in the form of a stool bank with a responsible scientific committee. The institution must have a doctor to evaluate, select and recruit stool donors; microbiologist and / or pharmacist to coordinate all procedures related to the processing and storage of feces; a biobank specialist to properly store faecal samples and a director to ensure compliance with all steps [30]. It is recommended that FMT be conducted as a treatment under investigation, within the framework of a scientific study and with signature of informed consent form by participants.

OBJECTIVE

The aim of this study was to describe the initial experience with fecal microbiota transplantation in the treatment of recurrent CDI in Brazilian cohort patients. We sought

to determine CDI resolution rate, occurrence of adverse events in short and long term and to evaluate factors related to therapeutic success.

METHODS

STUDY DESIGN

This is a prospective, open and uncontrolled pilot study in a single center, conducted at Instituto Alfa de Gastroenterologia, Hospital das Clínicas, Federal University of Minas Gerais (IAG-HC/UFMG), which sought to evaluate the effectiveness of fecal microbiota transplantation in patients with recurrent CDI between September 2017 and March 2020. Demographic data, clinical and laboratory variables, previous exposure to medications, duration of symptoms, and number of bowel movements per day were assessed. Stool shape was classified according to the Bristol scale and comorbidities using the Charlson comorbidity index [31,32]. The study was approved by local ethics committee (CAAE 72755217.8.0000.5149 – opinion 2.264.667 on 9/8/2017).

PATIENT POPULATION

Patients at least 18 years old, with recurrent *C. difficile* infection and who agreed to participate after signing the informed consent form were considered for inclusion. At enrolment, recurrent CDI was defined as the development of a new *C. difficile* infection within 8 weeks of a previous episode treated properly, in which there was an initial resolution of symptoms. Recurrence was characterized by the presence of diarrhea, with more than three daily excrements, with unformed stools (Bristol 6 or 7), in a minimum period of 48 hours, and microbiological confirmation for *C. difficile*. The laboratory approach was performed by a positive glutamate dehydrogenase (GDH) test (GDH ECO Teste - TR.0032 - Eco Diagnóstica, Minas Gerais, Brazil), followed by positive toxigenic culture.

Patients without laboratory confirmation were excluded, as well as pregnant, candidates under 18 years old, clinically ill with life expectancy less than three months, septic shock with hemodynamic instability and need for vasoactive drugs, and unable to sign

the informed consent form. Severe CDI was defined by the presence of one of the following criteria: bloody diarrhea, pseudomembranous colitis, adynamic ileus, severe abdominal pain, fever with axillary temperature over 38.9 °C, serum albumin below 2.5g/dL, global leukocyte count greater than 20,000 cells / mm^3 or acute renal failure. Complicated CDI was the infection that evolved with toxic megacolon, peritonitis, hemodynamic instability, respiratory failure or need for surgical treatment. Mild to moderate CDI was defined as the presence of diarrhea without additional criteria that characterize a serious or complicated condition.

DONOR STOOL PREPARATION AND FMT PROCEDURE

The source of donor stool was frozen samples obtained from our stool bank at the Fecal Microbiota Transplant Center of IAG-HC/UFMG. Donor selection, fecal sample preparation, storage, defrosting, pre and post-procedure care were the same as detail described in our previous report and were carried out in accordance with international guidelines on fecal microbiota transplantation and Brazilian epidemiological specificities.

All patients underwent FMT by colonoscopy after bowel lavage with polyethylene glycol (PEG) solution and 10 to 14 days of oral vancomycin regimen, as previously described. The quality of intestinal preparation was assessed using Boston Bowel Preparation Scale [33]. It was considered inadequate with a score between 0 to 3, regular between 4 to 5, and excellent/good between 6 to 9. All colonoscopies were performed by only one researcher.

OUTCOMES AND FOLLOW-UP

Post-FMT, all patients were monitored daily by telephone contact with approach to symptoms, occurrence of adverse events and assessment of diarrhea resolution. If serious side effects or persistent complaints were detected, patients were personally assessed by the researcher. After the first week, follow-up was done within eight weeks, three months, six months and, subsequently, annually to assess presence of diarrhea, use of antibiotics, hospitalization, development of new disease or complaint, and recurrence of CDI. Stool GDH test was performed whenever diarrhea occurred. If positive,

subsequent toxigenic culture was realized. Participants were instructed to contact the researcher on suspicion of recurrence of *C. difficile* infection or in the presence of any complaint or adverse event.

Adverse events were defined as any undesired occurrence after FMT, without the need for an exact causal relationship. Symptoms, disease onset or laboratory findings were considered. They were classified according to severity in *mild events* (mild symptoms, such as abdominal discomfort, diarrhea, constipation, flatulence, abdominal bloating, nausea, vomiting and fever with spontaneous resolution) or *major events* (perforation, bleeding, bronchoaspiration, transmission of pathogens, exacerbation of inflammatory bowel disease, occurrence of infection, need for hospitalization, temporary or permanent functional disability or death). Regarding the time of occurrence, they were classified as *short term* (within one month after FMT), *medium term* (between one month and one year) and *long term* (after one year). As for causality, they were classified into *definitely related* (there was a reasonable temporal sequence, with an expected response pattern and not explained by another hypothesis), *probably related* (there was a reasonable time sequence, with an expected response pattern and unlikely to be explained by the patients characteristics or other interventions), *possibly related* (despite the temporal relationship, it is possible that it is caused by factors other than transplantation) and *unrelated* (event that is certainly unrelated to treatment).

The main outcome of this study was to evaluate the efficacy and safety of FMT, calculating CDI resolution rate and occurrence of adverse events. CDI resolution rate was defined as disappearance of diarrhea related to *C. difficile* infection, or persistent diarrhea explicable by other causes with negative GDH and culture toxigenic at the end of eight weeks of treatment. The resolution rate can be primary if achieved with a single infusion or overall if new procedures are needed. FMT failure was defined as the recurrence of CDI within eight weeks after fecal infusion, characterized by more than three daily bowel movements, with unformed stools (Bristol 6 or 7) for more than 48 hours and laboratory confirmation by positive GDH and toxigenic culture. These patients were offered a new FMT with feces from another donor.

STATISTICAL ANALYSIS

Statistical analysis of the data was performed using the SPSS program (IBM Corp. Released 2013; IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). In the descriptive analysis, categorical variables were presented as frequencies and proportions. The numerical variables were presented as means and standard deviation or as medians and range when the distribution was not Gaussian. To assess the relationship between procedures that provided clinical remission with those that did not, with the rest of the variables, we considered Fisher's exact tests for the categorical and Mann Whitney tests for the continuous. Statistical significance was defined as $p < 0.05$.

RESULTS

Between September 2017 and March 2020, 91 candidates from 17 Brazilian states and federal district sought Fecal Microbiota Transplant Center of IAG-HC/UFMG to assess eligibility for transplantation (Figure 1). Of patients evaluated, 77 were excluded for not having recurrent CDI. Majority had chronic diarrhea (with no evidence of *C. difficile* infection), irritable bowel syndrome and inflammatory bowel disease. Patients with autism spectrum disorder, graft-versus-host disease (GVHD) after bone marrow transplantation (BMT), bullous pemphigus, depression, anxiety, celiac disease, ankylosing spondylitis, small intestinal bacterial overgrowth and food intolerance were also excluded. Ten patients received a presumptive diagnosis of recurrent CDI, but there was no laboratory confirmation. Four patients with recurrent CDI were contraindicated to FMT due to: refusal, minority, hemodynamic instability due to septic shock by pulmonary focus and palliative support in frail elderly. Of the 91 patients who were evaluated, only 10 were eligible for FMT.

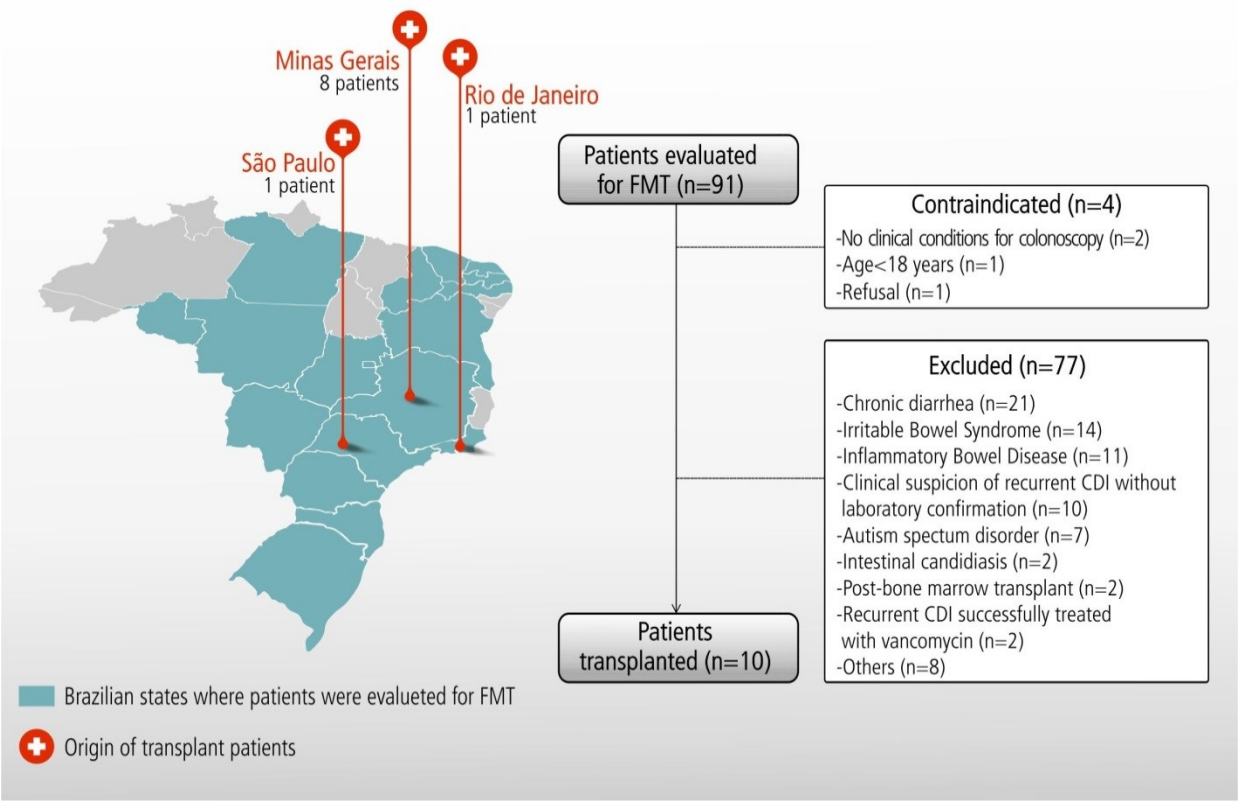

Figure 1. Panorama of patients evaluated for fecal microbiota transplantation.

Demographic data, comorbidities, risk factors and clinical characteristics of transplanted patients are summarized in Table 1. Of 10 patients included in the study, one lives in São Paulo, one in Rio de Janeiro and eight in Minas Gerais state. The majority were female 70% (7/10), median age was 68 (23 - 87) years and Charlson comorbidity rate was 3 (0 - 6). All had a history of recent antibiotic use, 60% were chronic users of proton pump inhibitors, half had been hospitalized before the first *C. difficile* infection and 40% had a previous history of malignancy. Two patients were on immunosuppressive therapy. The first was a 34-year-old man using tacrolimus 3 mg and dasatinib 100 mg due to graft-versus-host disease of liver, mouth and skin after bone marrow transplantation. The second was a 23-year-old man, using azathioprine 100 mg, prednisone 5 mg and ustequinumab 90 mg due to fistulizing Crohn's disease and overlap syndrome with autoimmune hepatitis and primary sclerosing cholangitis.

Table 1. Characteristics of 10 patients undergoing FMT

Variables	*n*	% (range)
Age in years, median	68	(23 – 87)
Female sex	7	70
Charlson comorbidity index, median	3	(0 – 6)
Previous neoplasia	4	40
Percentage of body weight loss, median	10	(2 – 20)
Albumin (mg/dL), median	3.1	(2.9 – 4.2)
WBC count (10^3/dL), median	8.4	(3.3 – 17)
Creatinine (mg/dL), median	0.9	(0.7 – 7.6)
Hospitalization before acquiring CDI	5	50
Antibiotic use before CDI	10	100
PPI usage	6	60
Recurrences of CDI, median	3	(1 – 4)
Positive toxigenic culture	10	100
Positive GDH	10	100
Positive toxin A/B test	6	60
Severity of CDI		
Mild/Moderate	4	40
Severe	6	60
CDI prior therapy		
vancomycin	2	20
metronidazole and vancomycin	7	70
fidaxomycin after metronidazole and vancomycin	1	10
Stool frequency per day, median	9	(5 – 17)
Time between first CDI and FMT in days, median	99	(51 – 212)

FMT, fecal microbiota transplantation; WBC, white blood cell; PPI, proton pump inhibitors; CDI, *C. difficile* infection

In all patients, a positive GDH test was confirmed by toxigenic culture before inclusion. Toxin A/B was positive in 60%. Majority had severe CDI at 60% (6/10) and was treated with metronidazole and vancomycin at 70% (7/10). The median of recurrent CDI was 3 (1 - 4) episodes. Stool frequency was 9 (5-17) bowel movements per day and stool consistency classified as Bristol 6 and 7. Median time between first CDI and FMT was

99 (51 - 212) days. Eighty percent were nutritionally-at-risk adults with a median involuntary loss of 10 (2 - 20) % of usual body weight within 6 months.

All transplants were indicated by recurrent CDI. There were no cases of refractory CDI. Eleven transplants were performed in 10 patients. Two candidates underwent intestinal preparation in hospital. The others performed at home. Source of all stools was unrelated donors. Average sample storage time was 39 (1 - 147) days, time between sample collection and storage was 3h42min (2h24min - 29h) and procedure duration was 16 (10 - 25) minutes. Intestinal preparation was considered excellent/good in 81.8% (9/11) and regular in 18.2% (2/11). The median amount of stool used was 295 (250 – 300) mL. After colonoscopy, patients remained in right lateral position for one hour. All were discharged on the day of FMT. Despite the loperamide used before the procedure, all patients eliminated a minimal portion of infused fecal substrate during the first 8 hours of follow-up.

Nine of 10 patients (90%) treated by FMT exhibited resolution of *C. difficile* infection. Primary resolution with single FMT was 80% and overall resolution after second FMT was 90%. Two patients did not respond to the first procedure. The median ICD recurrence was 9.5 days. One presented CDI recurrence on seventh day post-FMT and opted for vancomycin taper and pulse regimen. The other presented CDI recurrence 12 days after transplantation and received a new course of 125 mg oral vancomycin four times a day for 10 days and a new successful FMT. Comparing the procedures that provided clinical remission with those that did not, there was no difference in relation to donor employed ($p = 0.164$), quality of intestinal preparation ($p = 0.345$), CDI severity ($p = 0.273$), presence of comorbidities ($p = 0.809$), fecal volume used ($p = 0.618$), sample preparation time ($p = 0.478$) or storage time ($p = 0.814$). Similarly, there was no difference in the occurrence of adverse events in relation to successful transplants and those not. The characteristics between two groups are summarized in Table 2.

Table 2. Factors assessed between FMT that provided clinical remission and those with therapeutic failure

Variables		Failed FMT		Successful FMT		*p* value
		n (%)	median	n (%)	median	
Age in years, median			60.5		68	0.722
Female sex		1 (50)		6 (66.7)		0.618
Charlson comorbidity index, median			3.0		3.0	0.809
Previous neoplasia		2 (100)		3 (33.3)		0.182
WBC count (10^3/dL), median			7.9		8.6	0.478
Creatinine (mg/dL), median			0.8		0.9	0.408
Hospitalization before acquiring CDI		0 (0)		5 (55.6)		0.273
PPI usage		1 (50)		5 (55.6)		0.727
Percentage of body weight loss, median			10		10	0.906
Recurrences of CDI, median			3.6		3	0.457
Positive toxin A/B test		2 (100)		5 (55.6)		0.467
Severity of CDI	Mild/Moderate	0 (0)		5 (55.6)		0.273
	Severe	2 (100)		4 (44.4)		
CDI prior therapy	Van	0 (0)		2 (22.2)		0.200
	Met +Van	1 (50)		7 (77.8)		
	Met + Van + Fid	1 (50)		0 (0)		
Stool donor	Donor 1	1 (50)		0 (0)		0.164
	Donor 2	0 (0)		1 (11.1)		
	Donor 3	0 (0)		5 (55.6)		
	Donor 4	1 (50)		3 (33.3)		
Intestinal preparation	Excellent/good	1 (50)		8 (88.9)		0.345
	Regular	1 (50)		1 (11.1)		
Sample storage time in days, median			70		39	0.814
Time between sample collection and storage (h), median			16		3.7	0.478
Colonoscopy duration in minutes, median			17		16	0.812
Infused volume in mL, median			292.5		295	0.618
Follow-up time in days, median			591.5		426	0.346
Presence of adverse events		1 (50)		5 (55.6)		0.727

FMT, fecal microbiota transplantation; WBC, white blood cell; PPI, proton pump inhibitors; CDI, *C. difficile* infection; Van, vancomycin; Met, metronidazole; Fid; fidaxomycin; (h), in hours

Median follow-up time after transplantation was 432 (36 - 782) days. There were no major adverse events during the period. Mild adverse events were observed in 54.5% of procedures. Most events are probably related to FMT, of short duration, spontaneous resolution, without need for hospitalization. The two immunosuppressed patients did not experience any infectious adverse events. Details of each adverse event, occurrence and causality are described in Table 3. Regarding patient acceptance, 9 out of 10 stated that they would undergo a new FMT if necessary.

During the FMT of a 76-year-old female patient, with previous history of rectal cancer, a granular laterally spreading tumor located in cecum was diagnosed. The lesion was completely resected 3 months after the transplant. Histological evaluation showed villous adenoma with low grade dysplasia and free margins. The only patient who did not respond to treatment is an 87-year-old woman with a history of breast cancer and recurrent urinary tract infection, who underwent FMT after the 4th CDI episode. After therapeutic failure with FMT, a successful vancomycin taper and pulse regimen was chosen. After one year and nine months of follow-up, she developed a urinary infection by *Escherichia coli* and was treated with amoxicillin/clavulanic acid. In less than a month she developed a new episode of CDI and was treated with vancomycin.

Table 3. Occurrence of early adverse events per FMT

FMT	Adverse Events	Causality	Follow-up day
1	None	–	–
2	Hyporexia and bloating	Probably	day 2
3	None	–	–
4	Dehydration	Probably	day 1
5	Abdominal cramps; bloating	Probably	day 1 to 7; day 1
6	Abdominal discomfort; bloating	Probably	day 1 to 2; day 2 to 3
7	Abdominal pain	Probably	day 1
8	None	–	–
9	Fever, abdominal pain and nausea; diarrhea	Probably	day 1; day 1 to 7
10	None	–	–
11	None	–	–

FMT, fecal microbiota transplantation

DISCUSSION

FMT has a well-established role in the treatment of recurrent or refractory CDI. Currently, FMT is strongly indicated as a treatment option for second or subsequent recurrence of CDI [9]. The recommendation is based on the acceptable safety profile and proven efficacy in several randomized controlled trials and meta-analysis [30]. Meta-analysis conducted by Quraishi *et al*. confirmed the superiority of FMT over vancomycin in the resolution of recurrent or refractory CDI (RR: 0.23; 95% CI, 0.07-0.80) [34]. The overall clinical remission achieved by FMT was 92% (95% CI, 89% - 94%) and there was no difference regarding the use of fresh or frozen samples (92% vs. 93%, $p = 0.084$). Similarly, Ianiro *et al*. in a systematic review and meta-analysis of 1150 subjects treated with FMT, found a recurrent CDI resolution rate of 93% with multiple infusions and 76% with just one [35].

C. difficile infection is common in patients with inflammatory bowel disease (IBD) and is associated with increased mortality [6]. In this context, FMT is a therapeutic possibility, even in immunosuppressed patients [36]. Kelly *et al*. investigated FMT for CDI in patients with human immunodeficiency virus, post-organ transplantation, use of chemotherapy for cancer and inflammatory bowel disease under immunosuppressive treatment [36]. The CDI resolution rate was 78% in a single procedure and 89% after second. Even though it is a high risk group for opportunistic diseases, there are no reports of infectious complications related to FMT. However, 14% of IBD patients experienced exacerbation of intestinal inflammation. A similar finding was found by Khoruts *et al*. [37]. Up to 25% of IBD patients had a mild worsening of the inflammatory disease, with a cure rate of 74.4% [37].

Similarly, FMT appears to be safe and effective in recurrent CDI after bone marrow transplantation. Post-BMT individuals are predisposed to dysbiosis, with a nine times greater risk of developing CDI compared to other hospitalized patients [38]. Despite conventional antibiotic therapy, recurrence is common and therapy that reestablishes the intestinal microbiota is sometimes necessary. Small series of cases with recurrent post-BMD CDI indicates a resolution rate with fecal transplant of 85.7% and a good safety

profile in mean two-year follow-up [39]. Furthermore, there is the possibility of additional gain in improving GVHD and less need for immunosuppression in some cases. GVHD is a serious post-BMT complication that can affect various organ systems, especially the gastrointestinal tract. Acute intestinal GVHD, which does not respond to corticosteroids, is associated with an average annual survival of less than 30%. Initial studies point to the possibility of clinical and histological improvement of GVHD after multiple fecal transplants [40,41].

To best of our knowledge, this is the first complete report of a Brazilian cohort treated with FMT by colonoscopy, using frozen samples. FMT achieved a 90% clinical remission and the findings of this study are consistent with those reported in literature. In Brazil, until now, only one study had been reported [29]. Ganc *et al.*, in 2015, published a successful experience of FMT by infusing fresh samples via enteroscopy in 12 patients with recurrent CDI. Despite the growing number of CDI in Latin America, there are few reports on FMT [42-44]. Most are pilot studies with a small number of patients, without structuring a transplant center with a stool bank.

Interestingly, in the present study, a significant number of patients who sought our service did not have CDI laboratory confirmation. Despite the suggestive clinical presentation and presumptive diagnosis, patients started empirical antimicrobial treatment in their reference hospitals without laboratory assurance. Such practice makes evident the scarcity and unavailability of diagnostic methods in certain Brazilian regions. The implantation of a fecal transplant center is remarkable for allowing access to treatment that is not widely available, but there is still a lot to be done about epidemiological analysis and diagnostic measures in our country.

The previous structuring of a transplant center with a stool bank in our institution was a fundamental stage of the study. The Fecal Microbiota Transplant Center of IAG-HC/UFMG was structured within the scope of the institution's Tumors and Tissues Bank and approved by the institution's research ethics committee. The Tumors and Tissues Bank is a biobank in charge of collecting human biological material in an organized manner, collected and stored for research purposes, under institutional responsibility and management, without commercial purposes, according to national guidelines and regulations present in CNS Resolution 466/12, CNS Resolution 441/11 and Ordinance 2.201/2011 of the Ministry of Health and complementary.

Implantation of a frozen stool bank allows quick access to FMT, eliminates logistical barriers related to fresh stools and adds security by allowing traceability and monitoring of adverse events. The search for suitable donors requires prolonged time with a multi-person approach and extensive laboratory tests. When using frozen samples from previously selected donors, these limitations are overcome. Fecal samples are supplied regularly by approved donors and can be stored at -80 ºC for long periods without compromising safety or therapeutic response [45-47]. Randomized clinical trials show that the use of frozen faecal suspension is equally effective as a fresh suspension for the treatment of CDI [48-50]. Lee *et al.* evaluated 219 patients with recurrent CDI submitted to FMT via enema. The use of fresh and frozen samples resulted in a similar rate of clinical resolution (83.5% vs. 85.1%, $p = 0.01$ for noninferiority) with no difference regarding adverse events [49]. Recently, Jiang *et al*. found a similar CDI resolution among recipients of fresh or frozen samples by colonoscopy (87% overall resolution rate) reinforcing the applicability of frozen samples [50].

There is no administration route that is proven to be more effective than another. Meta-analysis observed a tendency towards higher efficacy rates with a lower gastrointestinal administration compared to upper, but without reaching statistical significance [51,52]. Among the lower route, enema was less effective than colonoscopy in recurrent CDI (66.3% vs 87.4%; $p < 0.001$) [52]. Although colonoscopy is more invasive and may be inappropriate for critically ill patients (as evidenced by two candidates in the present study), it was associated with higher cure rates (78% with single infusion versus 98% with multiple infusions) [51]. In addition, colonoscopy allows the infusion of a larger amount of fecal substrate and the identification of some risk factors for failure, such as pseudomembranous colitis or inadequate bowel preparation.

Although a single infusion is sufficient to achieve clinical remission, a considerable number of patients require multiple infusions. In this context, some studies indicate factors related to patient and procedure that may predict an inadequate response and the need for new procedures. Factors such as severe CDI and FMT in hospitalized patients predict need for a second treatment [53]. Other predictors described are surgery before FMT, female sex, low stool volume, pseudomembranous colitis, concomitant use of other antibiotics and previous hospitalization [8,54]. Ianiro *et al*. evaluated 64 patients with recurrent CDI who underwent FMT by colonoscopy [55]. Most were female, with

an average age of 74 years, 40% with severe CDI and 59% hospitalized. The remission rate with only one infusion was 69%. Severe CDI and inadequate bowel preparation were considered to be predictors of failure after single infusion. Such findings were not confirmed by the present study. Possibly, the small sample size was insufficient to assess predictors of therapeutic failure.

Our study reported an incidence of adverse events (AE) of 54.5%. All AE observed were mild, early and self-limited. As for causality, the reported symptoms were probably related to FMT. There were no deaths, hospitalizations or development of new disease during the follow-up period. However, it is not possible to attribute the occurrence of mild adverse events only to the microbiota transplant per se, since the complaints are also observed after bowel preparation and colonoscopy.

Bowel preparation with PEG-based solutions has a better safety profile compared to sodium phosphate preparations [56]. It is well tolerated, even in critical patients with comorbidities such as chronic kidney disease, congestive heart failure, or electrolyte imbalances. However, it can cause symptoms such as nausea, vomiting and abdominal fullness [56]. In addition, up to 33% of patients undergoing colonoscopy have minor adverse events, mainly abdominal discomfort and/or bloating [56]. Other symptoms reported after colonoscopy are self-limited gastrointestinal bleeding, nausea, heartburn, constipation, dyspepsia and diarrhea [57]. Symptoms are usually mild and resolve within a few days after the exam. Ko *et al.* reported a rate of mild adverse events of 34% within one week after colonoscopy [58]. Similarly, Park *et al.* also described occurrence of mild adverse events within 7 days in 20.9% of patients. The main complaint was abdominal pain followed by rectal bleeding and bloating [57].

Wang *et al.* in a systematic review assessed the incidence of adverse events in 1089 patients undergoing FMT [22]. Among them, 831 patients were treated for refractory or recurrent CDI. The overall incidence of adverse events was 28.0% in the CDI group. Besides that, the incidence of serious adverse events was 2.0% for the upper gastrointestinal tract and 6.1% for the lower tract. The most common symptom was abdominal discomfort followed by bloating, diarrhea, nausea, constipation and transient fever. However, the actual incidence of adverse events may have been underestimated by the fact that transient or mild AE can be ignored by researchers. In this sense, two pioneering studies on FMT found a higher incidence rate. Van Nood *et al.* showed an

adverse events incidence of 93.1% (27/29) [14]. FMT was performed by a nasoduodenal probe and main symptoms reported were belching, nausea, abdominal cramps, diarrhea, abdominal pain, infection, and dizziness combined with diarrhea. Cammarota *et al.*, in a randomized clinical trial for FMT by colonoscopy, founded an incidence of 94% (19/20) [17]. The main symptoms were diarrhea, bloating and abdominal cramps that disappeared in 12 hours. There were no serious adverse events.

Although there was no difference in the therapeutic response in relation to the donors used, donor 3 was responsible for more than half of the procedures and all were successful. Super-donor is a proposed term to describe individuals whose stools result in significantly more successful outcomes [59]. However, characterizing these donors is still a poorly understood task. Despite the absence of large-cohort based studies, the evidence suggests that donor's microbial diversity plays an influential role in therapeutic success of FMT [60]. Individuals who obtained a clinical response with FMT exhibited a more diverse microbiota compared to non-responders [60,61]. Effectiveness also depends on providing a microbiota capable of restoring metabolic deficits of receptor that contribute to the disease. Metabolic differences between responders and non-responders were investigated and an increase in butyrate production was associated with post-FMT CDI resolution [62]. Understanding the complexity of interactions between microbiota and host will be the key to better characterize donors and provide more targeted treatment.

Finally, the treatment acceptation found in this study was similar to that described in literature. Despite its unpleasant nature, acceptance of stool-based therapy is high. A study by Zipursky *et al*. found that up to 94% of patients undergoing FMT would accept a new procedure, if necessary [63]. Possibly, the justifying factor for this attitude is the favorable judgment between benefits/risks of the treatment in face of a debilitating disease with a great impact on patients' quality of life. Factors such as a high level of education and family support are crucial for better acceptance [64]. In addition, a relationship of trust between doctor-patient and medical experience is decisive in the indication of treatment.

The main limitation of this study is its small sample. However, this is a pilot study that implemented a new methodology in our institution and allowed access to a treatment unavailable until then. Clinical response and adverse events found here are similar to

those described in other studies. Another advantage was the methodological rigor, mainly in the selection of donors and patients. All patients had a confirmed microbiological diagnosis. The results were not influenced by bias in selection of asymptomatic *C. difficile* carriers or those with diarrhea due to different etiologies than CDI.

CONCLUSION

The treatment of patient with recurrent CDI is based on two pillars of equal importance: eradication of toxin-producing *C. difficile* and microbiota recovery. In this sense, the fecal microbiota transplantation appears as an important therapeutic option because it acts directly in the recovery of microbiota. In our small cohort, FMT was effective in treating recurrent CDI. The primary resolution rate with single FMT was 80% and overall resolution after second FMT was 90%, even in patients with severe CDI and multiple comorbidities. In addition, the occurrence of adverse events was similar to that observed in other studies, with no serious adverse event or transmission of infectious diseases. FMT also appears to be safe in immunosuppressed patients. Even in emerging countries, where there is concern about tropical and infectious diseases, FMT seems to be a good treatment strategy for recurrent CDI. Despite the relative heterogeneity in relation to methodology used worldwide, our findings with FMT by colonoscopy with frozen samples were similar to those previously related. Further prospective studies with a larger number of participants are needed to conclusively determine the efficacy and safety in Brazilian population.

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9. CONSIDERAÇÕES FINAIS

A infecção pelo *C. difficile* é uma importante causa de diarreia associada aos cuidados da saúde. Até o momento, poucos estudos avaliaram os aspectos clínico-epidemiológicos da ICD no Brasil, assim como a eficácia e segurança do TMF em países emergentes. A recente identificação do ribótipo hipervirulento 027 e estirpes produtoras de toxina binária no Brasil suscita alerta sobre a necessidade de melhoria nos métodos de enfrentamento à infecção pelo *C. difficile*. A criação de uma plataforma como a apresentada, capaz de atender casos graves e recorrentes de ICD, é de fundamental importância para enfrentar uma condição crescente no país e que foi responsável por grandes epidemias nos Estados Unidos e Europa nas últimas décadas.

Este estudo, até onde sabemos, foi o primeiro a descrever a experiência na implantação de uma unidade de transplante de microbiota fecal no Brasil. Ele procurou descrever instruções detalhadas para a estruturação de um centro de transplante fecal, como aspectos éticos e regulatórios, seleção de doadores, processamento e armazenamento de amostras, via de administração e acompanhamento pós-procedimento. As etapas de implantação descritas aqui devem facilitar a disseminação segura de centros de transplante fecal no país.

A estruturação de um centro de transplante fecal de microbiota com banco de fezes congelado permitiu o acesso a uma modalidade de tratamento inovador e que ainda não está amplamente disponível no país. O alcance do estudo foi nacional, com avaliação de pacientes provenientes de 17 estados da federação e do distrito federal. Esse comportamento explicita a carência de acesso ao TMF no território brasileiro e exalta o pioneirismo do presente estudo.

A seleção de doadores é um passo vital na estruturação de um centro de transplante. No entanto, encontrar doadores saudáveis e mantê-los fidelizados é uma tarefa desafiadora. Esse protocolo de seleção utilizou critérios clínicos amplos e foi capaz de identificar um grande número de contraindicações clínicas antes de empregar exames de sangue e fezes. A avaliação clínica rigorosa permitiu identificar contraindicações em possíveis doadores e racionalizar o uso de recursos.

Em nossa coorte, o TMF se mostrou eficaz no tratamento de ICD recorrente. A taxa de resolução primária foi de 80% e a taxa de remissão geral, após segundo procedimento, foi de 90%, mesmo em pacientes com quadros grave e múltiplas comorbidades. A ocorrência de eventos adversos foi semelhante à observada em outros estudos, sem eventos graves, óbitos ou transmissão de doenças infecciosas. O TMF também parece ser seguro em pacientes imunossuprimidos. Mesmo em países emergentes, onde há preocupação com doenças tropicais e infecciosas, o TMF parece ser uma boa estratégia de tratamento para CDI recorrente.

Apesar da heterogeneidade em relação à metodologia utilizada em todo o mundo, nossos achados com TMF por colonoscopia com amostras congeladas foram semelhantes aos relatados anteriormente. Mais estudos prospectivos com um número maior de participantes são necessários para determinar conclusivamente a eficácia e a segurança na população brasileira.

10. ANEXOS

ANEXO A

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Projeto: CAAE – 72755217.8.0000.5149

Interessado (a): Prof. Luiz Gonzaga Vaz Coelho
Depto. de Clínica Médica
Faculdade de Medicina- UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 08 de setembro de 2017, o projeto de pesquisa intitulado **"Centro de transplante de microbiota fecal do Instituto Alfa de Gastroenterologia do Hospital das Clínicas da UFMG: implantação e análise dos resultados em pacientes com infecção recorrente e refratária pelo clostridium difficile"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Dra. Vivian Resende
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – Cep:31270-901 – BH-MG
Telefax: (031) 3409-4592 - e-mail: coep@prpq.ufmg.br

ANEXO B

Arquivos de Gastroenterologia

Decision Letter (AG-2020-0100)

From: rviebig@gmail.com

To: albuquerqueterra@gmail.com

CC:

Subject: Arquivos de Gastroenterologia - Decision on Manuscript ID AG-2020-0100

Body: 30-Jun-2020

Dear Dr. Terra

It is a pleasure to accept your manuscript entitled "STRUCTURING A FECAL MICROBIOTA TRANSPLANTATION CENTER IN A UNIVERSITY HOSPITAL IN BRAZIL" in its current form for publication in the Arquivos de Gastroenterologia. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

We will get in touch with you soon to inform you about the edition.

Thank you for your fine contribution. On behalf of the Editors of the Arquivos de Gastroenterologia, we look forward to your continued contributions to the Journal.

Sincerely,
Dr. Ricardo Viebig
Associate Editor, Arquivos de Gastroenterologia
rviebig@gmail.com

Entire Scoresheet:

Date Sent: 30-Jun-2020

Close Window

11. APÊNDICES

APÊNDICE A

CENTRO DE TRANSPLANTE DE MICROBIOTA FECAL

FICHA DO DOADOR

INSTITUTO ALFA DE GASTROENTEROLOGIA
HOSPITAL DAS CLÍNICAS - UFMG

IDENTIFICAÇÃO **DATA** ____/____/_____

Nome:_____ Telefones: ( ) _____

Endereço:_____ ( ) _____

Sexo: _____Data de Nascimento: ___/___/___ Idade:_____Peso:_____Alt.: _____ IMC:_____

Profissão: _____ Estado civil:_____ Religião:_____

HISTÓRIA CLÍNICA

Registro HC:_____

Condições médicas pré-existentes

Lista de medicamentos de uso contínuo

CONTRAINDICAÇÕES

Sim	*Não*	
☐	☐	Infecção ativa não controlada durante a doação
☐	☐	Febre de origem desconhecida ou febre nas últimas 2 semanas
☐	☐	Exposição a antibióticos, imunossupressores ou quimioterápicos (últimos 3 meses)
☐	☐	Doença transmissível ativa (HIV, vírus da hepatite B, hepatite A e/ou hepatite C)
☐	☐	Exposição conhecida ou história prévia de HIV, HBV, HCV, sífilis, HTLV I e II, malária, tripanossomíase, tuberculose, herpes muco cutânea
☐	☐	Histórico de doenças gastrointestinais, incluindo doença inflamatória intestinal, síndrome do intestino irritável, doença celíaca, diarreia ou constipação intestinal crônica, neoplasias malignas gastrointestinais, procedimentos cirúrgicos gastrointestinais, excesso de gases, flatulência
☐	☐	História prévia de transplante de órgãos e tecidos (incluindo córnea)
☐	☐	História de transfusão sanguínea (últimos 6 meses)
☐	☐	História de acidente perfuro cortante (últimos 6 meses)
☐	☐	História recente (≤ 2 meses) de vacinação com vírus vivo atenuado em que é possível o risco de transmissão
☐	☐	Histórico de doenças autoimunes, atópicas ou terapia imunomoduladora em curso

☐ ☐ Fatores de risco para Doença de Creutzfeldt-Jacob (história pessoal prévia ou familiar, receptores de enxerto como transplante de córnea, uso prévio de hormônios de pituitária cadavérica, uso prévio de insulina bovina, exposição nosocomial, pessoas que permaneceram no Reino Unido e/ou na República da Irlanda por mais de 3 meses, entre 1980 e 1996 ou que tenha permanecido 5 anos ou mais, consecutivos ou intermitentes, na Europa após 1980 até os dias atuais)

☐ ☐ Profissionais da área de saúde expostos ao risco de transmissão de doenças infecciosas ou risco de serem carreadores de organismos com multirresistência a antimicrobianos

☐ ☐ Profissionais que trabalham com animais, sob risco de transmissão de zoonoses

☐ ☐ Comportamento sexual de alto risco (contato sexual com anônimos, contato sexual com profissionais do sexo, uso de drogas antes da relação sexual, contato sexual com indivíduos com HIV ou hepatites virais, homem que faz sexo com homem, múltiplos parceiros sexuais, trabalha como profissional do sexo ou história prévia de doença sexualmente transmissível) nos últimos 12 meses

☐ ☐ Novo contato sexual (últimos 12 meses)

☐ ☐ História prévia de doença sexualmente transmissível

☐ ☐ Uso prévio de drogas ilícitas endovenosas ou inalatórias

☐ ☐ História de encarceramento ou permanência por longo tempo em casas de repouso

☐ ☐ Implante de *piercing* ou realização de tatuagens (últimos 6 meses)

☐ ☐ História recente de hematoquezia ou outros sangramentos do trato gastrointestinal (últimos 6 meses)

☐ ☐ Doença diarreica aguda recente (últimos 6 meses)

☐ ☐ Sobrepeso e obesidade definidos pela Organização Mundial de Saúde como Índice de Massa Corporal (IMC) maior ou igual a 25 e 30 kg/m2 respectivamente

☐ ☐ Desnutrição moderada a grave

☐ ☐ Diabetes mellitus ou Síndrome metabólica

☐ ☐ Transtornos psiquiátricos

☐ ☐ Síndromes de dor crônica (fibromialgia, fadiga crônica) ou síndromes neurológicas

☐ ☐ Histórico de neoplasias malignas

☐ ☐ História familiar de Síndrome Polipoide ou Câncer Colorretal prematuro em parente de primeiro grau

☐ ☐ Uso crônico de inibidores de bomba de prótons (≥ 3 meses)

Exame Físico

Declaro que as informações acima são verdadeiras; Belo Horizonte, ___ de _____ de _____

_____ _____

Doador *Dr. Daniel Antônio de Albuquerque Terra*

APÊNDICE B

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado (a) a participar do estudo: *Centro de Transplante de Microbiota Fecal do Instituto Alfa de Gastroenterologia do Hospital das Clínicas da UFMG: implantação e análise dos resultados em pacientes com infecção recorrente e refratária pelo Clostridium difficile*. Este estudo objetiva avaliar a eficácia do Transplante de Microbiota Fecal (TMF) em pacientes com infecção pelo *Clostridium difficile* recorrente e refratária à terapêutica convencional com uso de antimicrobianos. O mesmo obteve aprovação do COEP/UFMG, órgão com o papel de esclarecer dúvidas ou questões éticas relacionadas à pesquisa.

Sua participação é voluntária e gratuita, ou seja, não haverá despesas ou remuneração. Ela constituirá em entrevista médica para avaliação do seu estado geral de saúde e aplicação de questionário específico com o objetivo de excluir afecções que contraindiquem a doação. Caso você participe, uma amostra de sangue será coletada em veia de seu braço para realização de exames. Serão exames gerais sobre seu estado de saúde e exames sorológicos para identificação de alguma infecção. Uma amostra de fezes também será coletada e estudada para afastar patógenos causadores de doença. Se você estiver apto para a doação, após essa triagem inicial, uma amostra de suas fezes será coletada e preparada para, posteriormente, ser transplantada em pacientes com infecção pelo *C. difficile* através de colonoscopia. Seu anonimato será mantido durante todas as etapas. A qualquer momento lhe será assegurada a possibilidade de desistência do projeto, sem qualquer prejuízo.

O benefício esperado está associado à probabilidade de cerca de 80 a 90% de melhora do quadro infeccioso de modo definitivo, em pacientes com infecção recorrente ou refratária pelo *C. difficile*. A coleta de fezes, após evacuação, constitui procedimento simples e isento de complicações. A coleta de sangue também constitui procedimento sem maiores complicações, podendo, às vezes originar dor leve ou pequeno hematoma no local da punção.

Eu,_____, carteira de identidade nº_____, li atentamente todo o texto acima e compreendi a natureza e objetivo do estudo do qual fui convidado a participar. A explicação que recebi menciona os detalhes da pesquisa, assim como seus benefícios e riscos.

1. Assim sendo, concordo com a doação voluntária de fezes para procedimento de TMF e que seja coletada uma amostra de minhas fezes para estudos futuros.
2. Consinto com a realização de exames de sangue para: HBV, HCV, HIV-1 e HIV-2, entre outros.
3. Concordo que todos os meus dados, como: idade, sexo, fatores relacionados, história familiar, exames laboratoriais, diagnóstico e tratamento possam ser retirados do meu prontuário médico.
4. Minhas informações, mantendo o anonimato, poderão ser usadas para publicação de resultados.

O participante e pesquisador assinarão duas vias iguais, ficando uma via com o participante e a outra com o pesquisador.

Belo Horizonte, ... de ..... de ....
Assinatura do Voluntário

Responsável Legal: ..... RG: ....
Assinatura

Testemunha: ..... RG: ....
Assinatura

Investigador Principal: .....
Dr. Luiz Gonzaga Vaz Coelho

Em caso de dúvidas ou qualquer outra informação que queira obter, entrar em contato com:

Dr. Luiz Gonzaga Vaz Coelho
Instituto Alfa de Gastroenterologia
Av. Professor Alfredo Balena, 110 – sl. 208.
Santa Efigênia. Belo Horizonte, MG.
CEP: 30130-100. Contato: (31) 3409-9403.
E-mail: lcoelho22@gmail.com

COEP - Comitê de Ética em Pesquisa
Av. Antônio Carlos, 6627. Unidade Administrativa II/sl. 2005.
Campus Pampulha - Belo Horizonte, MG - CEP: 31270-901
Contato: (31) 3409-4592 / coep@prpq.ufmg.br

APÊNDICE C

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você, paciente com diagnóstico de infecção por *Clostridium difficile* (CDI), está sendo convidado (a) a participar do estudo: *Centro de Transplante de Microbiota Fecal do Instituto Alfa de Gastroenterologia do Hospital das Clínicas da UFMG: implantação e análise dos resultados em pacientes com infecção recorrente e refratária pelo Clostridium difficile*. Este estudo objetiva avaliar a eficácia do Transplante de Microbiota Fecal (TMF) em pacientes com CDI recorrente ou refratária à terapêutica convencional com uso de antimicrobianos. O mesmo obteve aprovação do COEP/UFMG, órgão com o papel de esclarecer dúvidas ou questões éticas relacionadas à pesquisa.

Sua participação é voluntária e gratuita, ou seja, não haverá despesas ou remuneração. Ela constituirá em permitir que as informações sobre a sua doença contidas no seu prontuário médico sejam consultados pelos pesquisadores envolvidos e utilizadas na pesquisa, dentro do maior sigilo possível. Caso você participe, uma alíquota de fezes devidamente estudada e processada, uma vez afastados patógenos causadores de doença, será infundida em uma parte do seu intestino (cólon ascendente) por meio de colonoscopia. Durante o procedimento e imediatamente antes da infusão, será coletada uma pequena quantidade de suas fezes para estudos relacionados às bactérias que colonizam seu trato digestivo. Uma amostra de sangue será ainda coletada em uma veia de seu braço para realização de exames. Seu anonimato será mantido. A qualquer momento lhe será assegurada a possibilidade de desistência do projeto, sem qualquer prejuízo.

O benefício esperado está associado à probabilidade de cerca de 80 a 90% de melhora do quadro infeccioso de modo definitivo. A taxa de complicações relacionada à realização da colonoscopia é muito baixa (0,02%) e está associada, na maioria das vezes, à realização de biópsias ou retirada de pólipos, o que não será feito durante este procedimento. Este procedimento é acompanhado de sedação e você poderá sentir náuseas, desconforto ou mesmo dor abdominal. Tais sintomas também podem estar associados ao transplante de microbiota per si, mas não perduram por mais de 3 horas. A coleta de sangue também constitui procedimento sem maiores complicações, podendo, às vezes originar dor leve ou pequeno hematoma no local da punção.

Eu,_____, carteira de identidade nº_____, li atentamente todo o texto acima e compreendi a natureza e objetivo do estudo do qual fui convidado a participar. A explicação que recebi menciona os detalhes da pesquisa, assim como seus benefícios e riscos.

1. Assim sendo, concordo com o procedimento de TMF e que seja coletada uma amostra de minhas fezes e uma amostra de sangue para estudos futuros.
2. Consinto com a realização de exames de sangue para: HBV, HCV, HIV-1 e HIV-2, entre outros.
3. Concordo que todos os meus dados, como: idade, sexo, fatores relacionados, história familiar, exames laboratoriais, diagnóstico e tratamento possam ser retirados do meu prontuário médico.
4. Minhas informações, mantendo o anonimato, poderão ser usadas para publicação de resultados.

O participante e o pesquisador assinarão duas vias iguais, ficando uma via com o participante e a outra com o pesquisador.

Belo Horizonte, ... de ..... de ....

Assinatura do Voluntário

Responsável Legal: ..... RG: ....

Assinatura

Testemunha: ..... RG: ....

Assinatura

Investigador Principal: .....

Dr. Luiz Gonzaga Vaz Coelho

Em caso de dúvidas ou qualquer outra informação que queira obter, entrar em contato com:

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E-mail: lcoelho22@gmail.com

COEP - Comitê de Ética em Pesquisa
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Campus Pampulha - Belo Horizonte, MG - CEP: 31270-901
Contato: (31) 3409-4592 / coep@prpq.ufmg.br

APÊNDICE D

CENTRO DE TRANSPLANTE DE MICROBIOTA FECAL

INSTITUTO ALFA DE GASTROENTEROLOGIA
HOSPITAL DAS CLÍNICAS - UFMG

FICHA DE ACOMPANHAMENTO DO RECEPTOR

IDENTIFICAÇÃO

Nome:_____ Telefones: ( ) _____

Endereço:_____ ( )_____

Sexo: _____ Data de Nascimento: ___/___/___ Idade:_____ Peso:_____ Alt.: _____ IMC:_____

Hospital de Origem: _____ Prontuário:_____

HISTÓRIA CLÍNICA

Condições médicas pré-existentes

Lista de medicamentos pré-TMF (incluindo anti-ICD e IBP)

Alergias? ( ) sim ( ) não

Índice de comorbidade de *Charlson*: _____ %

HISTÓRIA DE INFECÇÃO PELO *Clostridium difficile* PRÉ-TMF

Data do diagnóstico inicial de ICD: ___/ ___/ _____ Duração dos sintomas antes do transplante _____ dias

Diagnóstico de ICD: ( ) Toxina ( ) GDH

Classificação da ICD

( ) Leve a moderada: *diarreia sem critérios adicionais que caracterizam quadro grave ou complicado.*
( ) Grave: *diarreia sanguinolenta, colite pseudomembranosa, íleo adinâmico, dor abdominal intensa, febre com temperatura > 38,9°C, hipoalbuminemia (albumina < 2,5g/dL), global de leucócitos ≥ 20,000 cel/mm³, insuficiência renal aguda.*
( ) Complicada: *megacólon toxico, peritonite, instabilidade hemodinâmica, insuficiência respiratória.*

Número de evacuações em 24h: _____ Escala de Bristol: 1( ) 2( ) 3( ) 4( ) 5( ) 6( ) 7( )

Exames pré-TMF (___/___/___):

Hb:_____ Htc:_____ GL:_____ (Bast.____Neut.____Eos____Linf____Mon____Bas____) Plqt: _____

Alb:_____ PCR:_____ Ur:_____ Crt:_____ Na:_____ K:_____ Mg:_____ HCO3:_____

RNI:_____ PTTa:_____ GJ: _____ Outros: _____

Dor abdominal? ( ) sim ( ) não Perda ponderal? ( ) sim ( ) não Perda estimada: _____ Kg

Número de tratamentos para ICD antes do TMF: _____

Se conhecido, qual o tratamento? ( ) Metronidazol ( ) Vancomicina ( ) Fidaxomicina

Indicação do TMF: ( ) ICD Refratária ( ) ICD Recorrente: ____ª recidiva

TRANSPLANTE DE MICROBIOTA FECAL

Data do TMF: ___/___/_____ Doador: _____

TMF realizado em paciente ( ) hospitalizado ( ) não hospitalizado Coleta de sangue? ( ) sim ( ) não

Local da infusão: ( ) Cólon direito ( ) Cólon esquerdo ( ) Outro Limpeza de casa com cloro? ( ) sim ( ) não

Volume da infusão: _____ Tempo início-fim colono: ___:___ > ___:___

Escala de Boston durante o TMF: _____ Tempo total:___:___

Loperamida pós-TMF? ( ) sim ( ) não

Trendelenburg por 1h pós-TMF? ( ) sim ( ) não

Complicação endoscópica durante o procedimento? ( ) sim ( ) não Qual:_____

Complicações no pós-TMF imediato? ( ) sim ( ) não Quais: _____

_____

AVALIAÇÃO COM 24h PÓS-TMF

Persistência da diarreia? ( ) sim ( ) não

Eventos adversos?

( ) Nenhum ( ) Náusea ( ) Vômito ( ) Febre ( ) Cólicas abdominais ( ) Diarreia ( ) Flatulência ( ) Infecção ( ) Distensão abdominal

( ) Constipação ( ) Sangramento intestinal ( ) Necessidade de hospitalização ( ) Dor abdominal ( ) Outros:_____

Observações

AVALIAÇÃO COM 1 SEMANA PÓS-TMF

Persistência da diarreia? ( ) sim ( ) não Resolução da diarreia ocorreu com quantos dias pós o TMF?_____

Eventos adversos?

( ) Nenhum ( ) Náusea ( ) Vômito ( ) Febre ( ) Cólicas abdominais ( ) Diarreia ( ) Flatulência ( ) Infecção ( ) Distensão abdominal

( ) Constipação ( ) Sangramento intestinal ( ) Necessidade de hospitalização ( ) Dor abdominal ( ) Outros:_____

Observações

RECORRÊNCIA PRECOCE DA ICD PÓS-TMF (≤ 8 SEMANAS)

Data da recorrência dos sintomas: ___/___/_____ Fez uso de ATB por outro motivo? ( ) sim ( ) não

Realizada limpeza do banheiro com agente esporicida? ( ) sim ( ) não

Número de evacuações em 24h: _____ Escala de Bristol: 1( ) 2( ) 3( ) 4( ) 5( ) 6( ) 7( )

Indicado nova infusão? ( ) sim ( ) não Data da nova infusão: ___/___/_____

Persistência da diarreia após a nova infusão? ( ) sim ( ) não

Resolução da diarreia ocorreu com quantos dias pós o novo TMF?_____

Obs: Caso esteja indicado um segundo TMF, o acompanhamento ocorrerá com 24h, 1 semana e 8 semanas.

AVALIAÇÃO COM 8 SEMANAS PÓS-TMF

Falha terapêutica? ( ) sim ( ) não

Presença de um dos seguintes critérios dentro de 8 semanas do TMF:
- persistência da diarreia: > 3 evacuações com fezes não formadas (Bristol 6 e 7) por 48h;
- necessidade de terapia adicional para ICD: segundo TMF;
- necessidade de colectomia;
- morte diretamente atribuída à ICD.

Eventos adversos?

( ) Nenhum ( ) Náusea ( ) Vômito ( ) Febre ( ) Cólicas abdominais ( ) Diarreia ( ) Flatulência ( ) Infecção ( ) Distensão abdominal

( ) Constipação ( ) Sangramento intestinal ( ) Necessidade de hospitalização ( ) Dor abdominal ( ) Outros:_____

Observações

AVALIAÇÃO COM 3 MESES PÓS-TMF

Eventos adversos?

( ) Nenhum ( ) Náusea ( ) Vômito ( ) Febre ( ) Cólicas abdominais ( ) Diarreia ( ) Flatulência ( ) Infecção ( ) Distensão abdominal

( ) Constipação ( ) Sangramento intestinal ( ) Necessidade de hospitalização ( ) Dor abdominal ( ) Outros:_____

Observações

AVALIAÇÃO COM 6 MESES PÓS-TMF

Eventos adversos?

( ) Nenhum ( ) Náusea ( ) Vômito ( ) Febre ( ) Cólicas abdominais ( ) Diarreia ( ) Flatulência ( ) Infecção ( ) Distensão abdominal

( ) Constipação ( ) Sangramento intestinal ( ) Necessidade de hospitalização ( ) Dor abdominal ( ) Outros:_____

Observações

AVALIAÇÃO COM 1 ANO PÓS-TMF

Eventos adversos?

( ) Nenhum ( ) Náusea ( ) Vômito ( ) Febre ( ) Cólicas abdominais ( ) Diarreia ( ) Flatulência ( ) Infecção ( ) Distensão abdominal

( ) Constipação ( ) Sangramento intestinal ( ) Necessidade de hospitalização ( ) Dor abdominal ( ) Outros:_____

Observações

AVALIAÇÃO COM 2 ANOS PÓS-TMF

Eventos adversos?

( ) Nenhum ( ) Náusea ( ) Vômito ( ) Febre ( ) Cólicas abdominais ( ) Diarreia ( ) Flatulência ( ) Infecção ( ) Distensão abdominal

( ) Constipação ( ) Sangramento intestinal ( ) Necessidade de hospitalização ( ) Dor abdominal ( ) Outros:_____

Observações

AVALIAÇÕES ANUAIS PÓS-TMF

Eventos adversos?

( ) Nenhum ( ) Náusea ( ) Vômito ( ) Febre ( ) Cólicas abdominais ( ) Diarreia ( ) Flatulência ( ) Infecção ( ) Distensão abdominal

( ) Constipação ( ) Sangramento intestinal ( ) Necessidade de hospitalização ( ) Dor abdominal ( ) Outros:_____

Observações

HISTÓRIA CLÍNICA PÓS-TMF

Alguma condição médica desapareceu após o TMF (p.ex. artrite, erupções cutâneas crônicas)?

( ) sim ( ) não Especifique:_____

Houve aparecimento de alguma condição médica nova após o TMF?

( ) sim ( ) não Especifique:_____