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Faecal Microbiota Transplantation (FMT) Therapy in Crohn's Disease:

Systematic Review

Short title: Faecal Microbiota Transplantation (FMT) Therapy in Crohn's Disease

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Disclosure

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Summary

Microbiota modulation strategies including faecal microbiota transplantation are finding an increasing place in the management of gastrointestinal diseases. This systematic review is the first to evaluate the efficacy of faecal microbiota transplantation in Crohn's disease.

Systematic review registration number: CRD42020163791

ABSTRACT

Background:

The gastrointestinal microbiota is the key antigenic drive in the inflammatory bowel diseases. Randomized controlled trials (RCTs) in ulcerative colitis have established faecal microbiota transplantation (FMT) as an effective therapy. We have conducted a systematic review to evaluate the efficacy of FMT in Crohn's disease.

Methods:

A systematic literature search was performed through to August 2020 [MEDLINE; Embase]. Studies were included if they reported FMT administration in patients with Crohn's disease, and reported on clinical outcomes.

Results:

Fifteen studies published between 2014 and 2020, comprising 13 cohort studies and two RCTs, were included in the analysis. The majority of trials evaluated FMT for induction of remission, with follow up duration varying from four to 52 weeks. One RCT in 21 patients, of single-dose FMT versus placebo, following steroid-induced remission, showed a higher rate of steroid-free clinical remission in the FMT group compared to the control group: 87.5% vs 44.4% at week 10 (P = 0.23). Another RCT, two-dose FMT in 31 patients, showed an overall clinical remission rate of 36% at week 8, however, with no difference in clinical or endoscopic endpoints between FMT administered by gastroscopy and colonoscopy. Considering all studies, the clinical response rates in early follow up were higher following multiple FMT than with single FMT. FMT dose did not appear to influence clinical outcomes, nor did whether FMT was fresh or frozen.

FMT delivered via upper gastrointestinal route demonstrated higher early efficacy rates of 75 to 100% compared with lower delivery route rates of 30 to 58%, but on follow-up beyond eight weeks this difference was not maintained. Whether pre-FMT antibiotic administration was beneficial was not able to be determined due to the limited number of patients receiving antibiotics and varying antibiotic regimens. No serious adverse events were reported.

Conclusions:

Preliminary studies suggest that FMT may be an effective therapy in Crohn's disease. However large controlled trials are needed. No serious safety concerns have been identified.

Key words:

Faecal microbiota transplantation; Microbiome; Crohn's disease; Therapeutics

INTRODUCTION

The gastrointestinal microbiota plays a critical role in Crohn's disease (CD) pathogenesis.[1, 2] An abnormal microbiota-host relationship, sometimes associated with predisposing genetic abnormalities, leads to excessive immune activation and subsequent intestinal inflammation.[3] Identifying the microbiota implicated in the pathogenesis of CD has focused on identifying novel pathogens as well as broad changes in microbial composition.[4, 5] Patterns of change in the gastrointestinal microbiota include reduced diversity, decreased representation of several taxa including *Faecalibacterium,* Ruminococcaceae and Bacteroides, and increase presence of Gammaproteobacteria and Fusobacteriaceae.[1, 6] A microbial signature associated with CD recurrence post-operatively has also been defined,[7] suggesting that the gut microbiota may be critical in promoting intestinal inflammation. To reverse some of these microbial abnormalities faecal microbiota transplantation (FMT) has been proposed as a treatment for CD.

The current standard of care in CD involves systemic immunosuppressive therapies. Less than half of all patients with CD achieve sustained remission, despite evidence-based best practice.[8] These therapies are associated with substantial morbidity and mortality.[9] FMT represents an alternative therapeutic approach, diminishing the antigenic drive, rather than suppressing the immune response.

FMT consists of the infusion of faeces from a healthy donor into the gastrointestinal tract of the recipient. Multiple randomised, placebo-controlled trials (RCTs) have demonstrated that FMT results in greater than 90% cure rates in recurrent C*lostridium difficile* infection (CDI).[10, 11] FMT has been shown to be therapeutically effective and safe in ulcerative colitis (UC). Five RCTs in adults, and one in children, have demonstrated that FMT effectively induces remission.[12-17] Stool from carefully screened healthy volunteers resulted in minimal side-effects. A further randomized controlled trial has demonstrated that FMT effectively maintains remission in UC.[18] Although the pathogenesis of CD differs from these conditions, they are associated with alterations in the microbiota.

Major questions remain about the optimal use of FMT in the treatment of CDI and UC, including frequency of treatment, dose, volume and route of administration. Several routes including naso-duodenal infusion, colonoscopic, enema and capsule formulation, at different doses and frequencies, have been found to be effective and safe for patients with both CDI and UC.[10, 11, 19]

The use of FMT in CD offers a broad and "non-selective" transformation of the entire microbiota. In this systematic review we aimed to evaluate the therapeutic efficacy of FMT in Crohn's disease.

MATERIALS AND METHODS

Protocol and Registration

This systematic literature search was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.[20] The research was registered (CRD42020163791) and published by PROSPERO on 28 April 2020, available from https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020163791.

Eligibility criteria

Eligible studies were case series, prospective cohorts, non-randomised and RCTs that investigated the use of FMT via any delivery modality in both paediatric and adult cohorts of patients with CD. Full text or abstract publications were included if they clearly described clinical outcomes including any of the following: 1) clinical response and efficacy; 2) biochemical, radiological or endoscopic response; 3) safety data; 4) quality of life measures. Animal and in vitro studies were excluded. Studies evaluating FMT in IBD were included if clinical outcomes in CD were separately presented. If multiple published articles included the same cohort of patients, they were all included, however abstracts reporting the same cohort were excluded. When duplicated data were included, the study with the most complete dataset was considered.

Search strategy

An electronic search of two databases [MEDLINE; Embase] was performed from inception to December 14, 2019. The search was re-run prior to final analysis (August 4, 2020). Back searching reference lists of all relevant papers was performed to identify additional studies. Abstracts from major meetings in gastroenterology were manually searched including The American College of **Gastroenterology** Annual Scientific Meeting (2017-2019), Annual Advances in Inflammatory Bowel Diseases conference (2017-2019), United European Gastroenterology Week (2017-2019), Digestive Disease Week (2017-2020), and European Crohn's and Colitis Organisation (2017-2019). International clinical trials registries were also searched to identify completed trial data including Australia New Zealand Clinical Trials Registry; US Clinical Trials Database, Health Canada Clinical Trials Database; ISRCTN registry; and the EU Clinical

Trials Register. The database search string used key words and phrases, in both free text and medical subject headings (MeSH), including alternatives of “Fecal Microbiota Transplantation”, “Crohn’s Disease” as well as clinical trial nomenclature, all of which were combined using Boolean terms (Supplementary material). The search strategy was limited to human subjects, but not by language. An additional search for systematic reviews across these indications was performed for bibliography review.

Study selection

Two investigators independently screened titles and abstracts of all articles identified in the search (SF, CB). Full texts of articles were reviewed according to eligibility criteria for inclusion in the analysis.

Data collection

Data were extracted from eligible studies into a standardised form that included study and patient characteristics, FMT characteristics including pre-FMT therapy, dose, treatment frequency, mode of delivery and donor details. Clinical endpoints, adverse events and results were documented.

Risk of bias in individual studies

Two investigators (SF, CB) independently assessed methodological quality and bias by using two assessment tools according to the type of study.[21, 22] The Cochrane Collaboration’s ‘risk of bias’ tool was used to assess RCTs.[22] In studies without a control group, a published modified version of The Newcastle-Ottawa Score was used.[21] The inter-investigator agreement was 100% (Cohen’s kappa = 1) for the RCTs, and 100% (Cohen’s kappa = 1) for the cohort studies. There was no need for discrepancy resolution by consultation with a third investigator.

Synthesis of results

According to The Cochrane Collaboration’s ‘risk of bias’ tool, overall judgement of the risk of bias was classified as “low” or “high,” based on the number of domains at risk of bias, and subjective evaluation of their respective risk. For the modified Newcastle-Ottawa Score we assigned “low”, “medium”, or “high” based on quality scores of 1-3, 4-5, 6-8, respectively. Given the small number of studies available for

inclusion, and heterogeneity amongst them, systematic analysis was reported in narrative form. This framework included clinical response and remission rates, as well as studies analysis robustness and an exploration of the relationship between these studies.

RESULTS

Study selection

Of 134 studies identified through the initial search, 15 studies published between 2014 and 2020 were included in the final analysis (Figure 1).[23-37] Study features and patient characteristics are shown in Table 1. This included two RCTs[23, 24] and 13 prospective cohort studies.[25-37]

All abstracts had subsequently been published in journals and therefore were excluded from analysis.

All studies were performed in single centres apart from one RCT performed across six centres in France.[24] The other RCT was performed in China,[23] as were seven cohort studies.[25-31] The remaining studies were conducted in the USA,[32-35] Belgium,[36] and Poland.[37] There were three paediatric cohorts;[32, 34, 37] the remainder were adult trials. Ten studies included patients with CD only,[23-28, 30, 32, 33, 36] the remaining five included other IBD subtypes but the results were separated.[29, 31, 34, 36, 37]

Six publications examined data from the same clinical trial in China (NCT01793831);[25-28, 30, 31] only the most recently published study with the largest dataset was included in our results.[30] Therefore ten studies were analysed including a total of 293 patients.

Study characteristics

Characteristics of included patients are shown in Table 1. FMT protocols and endpoint definitions were separated according to cohort studies and RCTs, in Tables 2 and 3, respectively.

Study Results

FMT preparation

All studies performed single donor FMT. The homogenization process described in all studies was stool blending in ambient air. Stool preparation and storage was fresh (n=6), frozen (n=3) and both (n=1). The type of FMT preparation did not appear to influence clinical response or remission rates across the studies.

Xiang et al initially used fresh or frozen FMT repeatedly until a response was achieved. Clinical response from a single FMT was 75.3% (131 / 174) at 1 month, at which time frozen FMT had lower clinical efficacy when compared to fresh FMT (difference 11.3%).[30] Based on this finding, fresh FMT was encouraged thereafter resulting in clinical response rates of 43.7% (76 / 174) after single or multiple FMT treatments at final follow up (median number of months was not specified).

High response rates (43% - 100%) were reported in the three paediatric cohorts using fresh (n=2) and frozen (n=1) FMT, however sample size was small in these studies.[32, 34, 37]

The only study demonstrating endoscopic improvement used fresh FMT.[24]

FMT dose

Five cohort studies and one RCT reported treatment with a single FMT treatment; in the remaining studies (n=4) FMT was administered repeatedly (2 - 8 treatments) across a wide time interval of 1 day to 6 months.

The likelihood of achieving clinical remission did not appear to differ according to receipt of single or multiple FMT. Remission rates for both single or multiple infusions ranged between 0% and 100%.[23, 32, 35] However, clinical response rates in the early follow up period (2 – 4 weeks) of 80 - 100% post multiple FMT [23, 37] were higher than that of single FMT trials ranging between 30% and 75%.[33-35]

Xiang et al's subgroup analysis compared single and multiple FMT treatments (median 3.5 FMT sessions), demonstrating an improved remission rate with multiple FMT treatments 23 / 109 (21.1%) compared to a single FMT 12 / 131 (9.2%).[30] Vermiere et al had no patients achieving remission after two FMTs, although the interval was only one day.[36]

Paediatric cohorts described remission rates of 56% and 29% following a single FMT treatment at three and six months, respectively.[32, 34]

One RCT that included 17 patients described corticosteroid-free remission rates of 87.5% at week 10 and 62.5% at week 20 after single-dose FMT. However, FMT was administered following induction of clinical remission with corticosteroids.[24]

Studies examining FMT dosing have been divided into those using <50g of stool per FMT (n=3),[32, 35, 37] and >50g of stool per FMT (n=5).[23, 24, 29, 34, 36] The amount of faecal material infused did not appear to influence treatment efficacy. The highest dose was 200g in Vermeire and Yang's studies of 33 patients, administered on two occasions using upper and lower gastrointestinal routes. Week 8 remission rates were 0 and 36%, respectively.[23, 36] In two studies using single FMT at lower doses (30 - 100g) remission rates at weeks 10 - 12 were higher (56% - 87.5%).[24, 32]

Route of delivery

Studies that utilised upper (n=3)[29, 32, 37] and lower (n=3)[24, 33, 35] gastrointestinal routes of delivery were analysed. The upper routes of delivery included infusion via gastroscopy, naso-gastric or jejunal tube, and mid-gut or naso-jejunal transendoscopic enteral tubing (TET). Lower gastrointestinal routes included colonoscopy, rectal tube and colonic TET. Four trials administered FMT via combinations of upper and lower routes. No trials used FMT delivered by capsule or enema. A parallel arm RCT compared FMT via gastroscopy with colonoscopy and found no significant differences in clinical or endoscopic efficacy.[23]

In the studies examining only one route of delivery, including the study by Xiang et al that administered FMT via upper routes in all patients except for one who received FMT via colonic TET, patients treated by the upper route experienced early (< 8 weeks) clinical response rates of 75 to 100% compared with lower delivery route rates of 30 to 58%.[30, 33, 35, 37] Similarly, remission rates were higher amongst patients receiving FMT through upper routes.[32, 37]

Beyond eight weeks, no route of induction FMT delivery showed clear benefit.

Suskind et al administered a single FMT infusion via nasogastric tube, resulting in a clinical remission rate of 56% at week 12.[32] Xiang et al, administered FMT mainly via upper routes; response and remission rates at final follow up were 76 / 174 (43.7%) and 35 / 174 (20.1%), respectively.[30] In a cohort of patients with long standing CD refractory to standard therapy a response rate of 32% (10 / 19) at week 12 was reported after receiving single dose FMT via colonoscopy.[35]

The remaining studies included both upper and lower routes of FMT delivery and reported clinical efficacy of less than 45%.[23, 34, 36]

Adjunctive therapies

Twenty-two patients in four studies received pre-FMT antibiotic administration;[29, 32-34] however no studies used the same regimen.

In two studies involving 12 patients, rifaximin was given orally three times per day for three days prior to FMT in a dose of 550mg[33] or 200mg[32]. Clinical response occurred in one out of three patients (33%) in the former study but no patient demonstrated an endoscopic response.[33] In the latter study of 9 patients clinical remission was seen in 78% at week 2 and 56% at week 12.[32]

In the study by Wei et al, vancomycin was administered at a dose of 500mg orally twice per day for three days until 12 hours prior to FMT, resulting in no improvement in the IBDQ score.[29] One study used metronidazole or vancomycin at 10 mg/kg/dose (maximum dose of 500 mg) three times a day for five days until two days prior to FMT, six month clinical response and remission rates were 3 of 7 (43%) and 2 of 7 (29%) respectively.[34]

Among all reviewed studies there was great variability in concomitant medication administered as part of the FMT procedures including antiemetics, anti-reflux medication, use of bowel lavage, and anti-peristaltic therapies.

Therapeutic Outcomes

Clinical response and remission rates, and endoscopic endpoints, are detailed in Table 4.

Two RCTs were included in this study evaluating clinical and endoscopic endpoints.[23, 24] Three other studies reported endoscopic endpoints.[33, 35, 36] One study evaluated a radiological endpoint.[26]

In the study by Sokol et al 21 patients with long-standing active Crohn's disease, predominantly ileocolonic, were randomised after achieving clinical response within three weeks of corticosteroid induction therapy.[24] Corticosteroids were tapered according to a predefined schedule (decrease by 10 mg every week until 50%

of the initial dose and then decrease by 5 mg per week until complete cessation) and no other CD drug therapies were permitted. Seventeen patients completed the study. Following bowel lavage, a single dose was delivered by colonoscopy of 50 - 100g of fresh stool from a healthy unrelated donor (n=8) or sham FMT (n=9). The primary endpoint of recipient colonization by donor microbiota was not achieved in any patient.

Rates of steroid-free clinical remission were 87.5% for FMT versus 44.4% for Sham FMT at week 10; and 62.5% versus 33.3% at week 20; neither of these differences were statistically significant. The endoscopic outcome (CDEIS) decreased significantly at week 6 following FMT (pre-FMT median 8.5 [IQR 4.6 - 13.0] vs. post-FMT 3.5 [IQR 1.0 ; 8.9]; p = 0.03), while no change was observed in the sham group (pre-FMT median 2.4 [IQR 0.0 ; 8.3] vs. post-FMT 2.7 [IQR 0.7 ; 10.0]; p = 0.8). Baseline CDEIS scores were lower in the sham group.[24]

In the controlled trial by Yang et al 31 patients received two doses, separated by one week, of fresh stool by gastroscopy or colonoscopy.[23] Each dose consisted of 200g of fresh stool from healthy related or unrelated donors. Patients with mild to moderate colonic CD were included and allowed to continue stable doses of CD drug therapy. The gastroscopy group received pre-FMT omeprazole and metoclopramide post-infusion. The colonoscopy group underwent bowel lavage and received loperamide prior to infusion. Four patients, two from each group, withdrew from the study.

Clinical remission was achieved in nine of 27 patients (36%). Remission rates were greatest at week 2 (67%), at which time no significant difference was seen between the gastroscopy and colonoscopy groups: 69.2% versus 64.3%, respectively. No significant endoscopic response or remission was achieved in either group.[23]

In the study by Vermiere et al, no clinical or endoscopic response was seen in the six patients with Crohn's disease; however this was not the same for patients with UC.[36]

He et al reported a cohort of 25 patients with moderate to severe CD complicated by an abscess or phlegmon on MRI or CT scan. Healing of the abscess occurred in 9.5%, improvement in 71.4%, no change in 9.5%, and worsening in 9.5%.[26]

Safety

Adverse events were monitored and reported in most studies (Table 5). There were no reports of serious adverse events related to FMT.

Risk of bias within studies

Nine out of 13 cohort studies had medium quality scores, three were considered high quality, one considered low (Figure 2). All studies adequately selected patients and ascertained the exposure and outcomes. None of these studies excluded alternative explanations for the effect due to receipt of concomitant medications. Follow up ≥ eight weeks was considered acceptable. All but one study was deemed to have sufficiently detailed description to allow for research replication. Risk of bias for the two RCTs was deemed high, relating to patient selection, performance and detection (Figure 3).

DISCUSSION

This is the first systematic review focussed on evaluating FMT in Crohn's disease. This study has revealed the therapeutic potential of this treatment but has also provided insight into the limitations of the literature. Published data include a small number of RCTs and comprise predominantly non-comparative cohort studies. We identified 15 studies, 13 cohort studies and two RCTs. Broad study eligibility criteria were utilised in order to widen the identification of trials; however, the inclusion of non-comparative cohorts has increased the potential for overestimating efficacy. No meta-analysis was performed in this review due to the further risk of biases with pooled estimates.

Previous systematic reviews of FMT in IBD have performed subgroup analyses of CD patients demonstrating remission rates ranging from 30% to 60%, with a large element of heterogeneity.[38-40] This review found modest clinical efficacy when adjunctive FMT was used for Crohn's disease. Sokol et al excluded patients receiving CD drug therapy and mandated a corticosteroid taper.[24] Three trials excluded patients receiving biologic agents, allowing other CD therapies to continue.[29, 32, 35] Xiang et al required cessation of all CD drug therapy at trial commencement, however their step-up FMT approach incorporated use of exclusive enteral nutrition and corticosteroids, azathioprine, or thalidomide.

Clinical remission rates were reported in nine studies, and response rates in eight, at varying follow up time points, making direct comparison difficult. Additionally, the FMT protocols differed substantially, even for

patients in the same study. The variable protocols reflect the absence of universal guidelines for FMT in terms of preparation, route of delivery, dosing and frequency.

Xiang et al described a significant difference between fresh versus frozen FMT; the remaining studies did not identify a consistent advantage of either preparation.[30] A recent study demonstrated a reduction in overall viable microbiota composition after freeze-thawing that was not significantly different to fresh anaerobically prepared specimens.[41] A recent meta-analysis has directly compared fresh FMT to frozen FMT in CDI, concluding that there was no significant difference in cure rates between the two methods (RR = 0.42; 95%CI: 0.05–3.94; *P* = 0.45),[11] consistent with other reviews.[10] Subgroup analyses from a meta-analysis of six RCTs comparing different modes of FMT in UC described a significant difference when comparing frozen FMT to placebo (OR = 2.76, 95% CI 1.59–4.79, P = 0.0003). The two studies that included both fresh and frozen FMT compared to placebo were not significant, however this may reflect the use of a single donor and different modes of delivery.[19]

No conclusions can be drawn about differences in efficacy related to mode of faecal processing, as all studies utilised an aerobic homogenisation technique. Recent studies have demonstrated a profound impact of ambient air processing on bacterial composition viability.[41, 42] Aerobic processing affects the viability of oxygen sensitive species including anaerobic commensals such as butyrogenic Faecalibacterium prausnitzii and *Euobacteriam halii*. These organisms have particular significance in Crohn's disease.[41, 43]

It was not possible to determine adequately the effect of FMT frequency, dose or single versus multi-donor FMT. Only single donor FMT was used in all studies. Multi-donor FMT has demonstrated greater therapeutic benefit than single donor in UC.[12-15, 17]

Early response rates were higher in two studies that administered multiple FMTs.[23, 37] Several studies did not specify the number or frequency of FMT treatments. Subgroup analyses from systematic reviews evaluating FMT in CDI have demonstrated increased efficacy associated with multiple FMTs.[10, 11] Ianiro et al showed a lower efficacy rate when a single infusion of less than 50g was administered, however dosing had no impact on efficacy with multiple infusions.[10] Similarly, repeated FMT dosing in UC in multiple RCTs has demonstrated greater efficacy than when one or two infusions are performed.[12-15, 17]

The optimal route of FMT depends on patient factors including clinical indication, anatomic location of disease, presence of co-morbid disease, and practical access to FMT. In the treatment of CDI all

modalities have demonstrated comparable efficacy of greater than 80%, the greatest efficacy appearing to relate to colonoscopic infusion.[10] The optimal route of delivery in UC is less certain; different routes at various frequencies have been found to be effective and safe.[12-15, 17] In the current review several studies utilised multiple routes of delivery without performing subgroup analyses to differentiate efficacy according to route of administration. Six studies examined only upper or lower delivery, demonstrating improved early efficacy with the former, although beyond eight weeks after treatment no route of administration showed superiority. Frequent small bowel involvement in Crohn's disease may explain higher efficacy early in FMT when it was delivered to the upper gut as opposed to colonoscopic delivery. However Yang et al, found no significant difference in clinical or endoscopic outcomes when directly comparing gastroscopy and colonoscopy routes in patients with colonic CD.[23] The mode of FMT delivery for small bowel disease needs to be assessed further.

Transenteral endoscopic tubing is a convenient strategy for repeated FMT and can be performed via both upper and lower routes,[44, 45] However, this has only been evaluated in one study, without subgroup analyses published.[26-28, 30] FMT in capsule form is more convenient, and enemas may be suitable for patients with Crohn's proctitis, however neither modality has been studied in CD.

Pre-FMT administration of antibiotics for the recipient reduces the microbiota richness and diversity, theoretically creating a "biological niche" for FMT, increasing colonization by donor microbiota in animal models,[46, 47] and possibly increasing remission rates in human recipients.[48] In this systematic review, no benefit of pre-FMT antibiotic administration was identified, but the number of patients receiving antibiotics was small and antibiotic regimens differed greatly.

Patient selection is likely to play an important role in achieving success with FMT therapy in CD due to the heterogenous nature of the condition. The majority of studies did not stratify for disease location. The inclusion criteria varied significantly allowing for participation based on clinical disease activity ranging from mild to moderate, moderate to severe, or any disease activity. The majority of studies did not specify whether they included patients with fistulising disease or abscess at the time of enrolment, although five studies included patients with these complications.

In this systematic review, no serious adverse events were reported. The majority of adverse events were self-limiting gastrointestinal symptoms or fever. This is consistent with previous meta-analyses evaluating FMT in IBD.[19, 40] A meta-analysis of side effects in six studies evaluating FMT in UC found no significant difference in incidence of adverse events between FMT and placebo groups.[19] The risk of worsening IBD

activity following FMT has also been examined, according to a subgroup analysis that included only high-quality studies the risk was considered marginal.[49] FMT has been shown to be safe and well tolerated in paediatric cohorts.[34]

In terms of donor profile and selection, all studies used single donor FMT. Multi-donor FMT in UC has demonstrated efficacy,[13, 15] however there are no comparisons of single versus multi-donor FMT. Most trials use individual donor FMT to enable strain tracking for safety reasons. In the studies in this review only limited information was provided regarding stool donor sources, at most only donor age and gender being described.

The optimal FMT donor is yet to be determined however, the "superdonor" phenomenon has been suggested in UC and irritable bowel syndrome.[13, 14, 50] Specific bacterial taxa associated with response or lack of response to FMT in UC have been identified;[15, 51] further studies using shot gun metagenomic sequencing may allow identification of donor organisms that are responsible for efficacy. This has the potential to engineer more personalised therapy.

This systematic review has several limitations. There are limited published studies, and those that exist vary widely in study design. Cautious interpretation of the results is warranted due to overrepresentation of low methodological quality studies. The studies reported may suffer from selection, ascertainment and detection biases. However, the majority were considered medium quality cohort studies. There were only two comparator studies included. Not all studies utilised standardised clinical indices that have been validated for assessing clinical response and remission, impairing comparisons.

CONCLUSION

Given the limitations of reported studies, there appears to be therapeutic potential for the use of FMT in CD. More randomised trials with objective disease activity assessment via endoscopy, biomarkers and non-invasive imaging methods such as intestinal ultrasound would improve our evaluation of the therapeutic efficacy of FMT in CD.

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Table 1. Patient and study characteristics

Study	Year	Country (centres)	Study level	CD sample size	IBD type	Cohort	Inclusion criteria	Severity	Duration of disease	Concomitant therapy
Cui et al[25]	2014	China (1)	Prospective noncomparative cohort study	49	CD	Adult	Moderate to severe CD; HBI ≥ 7	HBI: 11.7 ± 4.5	7.4 ± 5.3 years	All CD drug therapy was ceased.
He et al[26]	2017	China (1)	Prospective noncomparative cohort study	25	CD	Adult	Moderate to severe CD complicated by abscess/phlegmon on MRI/CT; HBI ≥ 7	HBI: 11 ± 2.68	6.2 ± 3.91 years	All CD drug therapy was ceased.
Li et al[27]	2019	China (1)	Prospective noncomparative cohort study	69	CD	Adult	Active CD; HBI score > 4 despite standard treatment; Clinical response to FMT	HBI: 1st FMT 8.51 ± 2.55, 2nd FMT 5.48 ± 2.92	7 ± 5.48 years	All drug CD therapy was ceased prior to first FMT.
Wang et al[28]	2018	China (1)	Prospective noncomparative cohort study	156	CD	Adult	Mild to severe CD; HBI > 7	HBI: 9	5 years	NS
Xiang et al[30]	2019	China (1)	Prospective noncomparative cohort study	174	CD	Adult	CD with any therapeutic target	HBI: 8 (6-10)	5 (2-9) years	All CD drug therapy was ceased. Commenced corticosteroids ± azathioprine / thalidomide / exclusive enteral nutrition as part of 3rd stage of step up FMT.
Zou et al[31]	2019	China (1)	Prospective noncomparative cohort	11	CD/ UC	Adult	Moderate to severe CD; HBI ≥ 7	NS	NS	Exclusion criteria: immunomodulators or corticosteroid use.

			study							
Wei et al[29]	2015	China (1)	Prospective noncomparative cohort study	3	CD/ UC	Adult	Mild to moderate CD; CDAI score of >150 and <400; C-reactive protein >10mg/L	CDAI: 345.00 ± 77.78	2.5 years	Stable doses of 5-ASA or corticosteroid continued. Exclusion criteria: anti-TNF agent within 2 months.
Yang et al[23]	2019	China (1)	Randomised, double blinded, parallel two-arm trial	31	CD	Adult	Mild to moderate CD; CDAI > 150; Colonic disease	CDAI: Group 1 275 ± 128, Group 2 290 ± 134; SES-CD: Group 1 4.87 ± 0.89, Group 2 5.13 ± 0.83	Group 1: 14.8 ± 3.8; Group 2: 15.4 ± 4.7 months	Stable doses of 5-ASA, thiopurines or corticosteroid continued.
Vaughn et al[35]	2016	USA (1)	Prospective noncomparative cohort study	19	CD	Adult	Active CD; HBI ≥ 5; > 3 years duration; Refractory to standard therapy	NS	12.5 (10.6) years	Stable doses of 5-ASA or thiopurines continued. Corticosteroids tapered to 20 mg of prednisone. 12-week washout for cyclosporine, tacrolimus and biologic agents.
Gutin et al[33]	2019	USA (1)	Prospective noncomparative cohort study	10	CD	Adult	Active CD; HBI ≥ 3	HBI 8.2 ± 4; SES CD: 8.2 ± 6.2	15.8 ± 14.1 years	Stable doses of CD drug therapy continued.
Vermeire et al[36]	2016	Belgium (1)	Prospective noncomparative cohort study	6	CD/ UC	Adult	IBD; Refractory to immunomodulators	CDAI: 290 (243-359); CDEIS 11.8 (9.5-17.2); SES-CD 17.5 (17-19.5)	14.67 years	CD drug therapy continued.
Sokol et al[24]	2020	France (6)	Randomised, single-blind, placebo-controlled trial	21	CD	Adult	Active CD at screening (HBI > 4); Clinical response to corticosteroid induction (HBI <5)	HBI: 2.0 (0.0-3.0); CDAI 62.0 (41.0-109.0); CDEIS 4.6 (0.2-10.5)	9 (5-15) years	Corticosteroids were tapered. Exclusion: anti-TNF agent within 1 month, immunosuppressants within 3 months.

Karolewska-Bochenek et al[37]	2018	Poland (1)	Prospective noncomparative cohort study	2	CD/UC	Paediatric	CD or UC; Refractory to standard therapy; Colonic disease	PCDAI: 30	2.75 years	Stable doses of CD drug therapy continued.
Goyal et al[34]	2018	USA (1)	Prospective noncomparative cohort study	7	CD/UC/IC	Paediatric	Mild to moderate IBD (CD, UC, IC); PCDAI 10-40; Biomarkers > x2 upper limit	PCDAI: 22.5; SES-CD: 12	3 (0.6-10) years	Stable doses of CD drug therapy continued.
Suskind et al[32]	2015	USA (1)	Prospective noncomparative cohort study	9	CD	Paediatric	Mild to moderate CD; PCDAI 10-29	PCDAI 19.7 ± 7.2	3.9 ± 1.8 (0-7) years	Stable doses of CD drug therapy continued. Exclusion criteria: prior use of biologic agent.

CD: Crohn's disease; HBI: Harvey-Bradshaw index; MRI: magnetic resonance imaging; CT: computerised tomography; FMT: faecal microbiota transplantation; UC: ulcerative colitis; IBD: inflammatory bowel disease; CDAI: Crohn's disease activity index; CDEIS: Crohn's disease endoscopic index of severity; SES-CD: simple endoscopic score for Crohn's disease; PCDAI: paediatric Crohn's disease activity index; IC: indeterminate colitis.

Table 2. FMT protocols and endpoint definitions in cohort studies

Study	Pre-FMT medications	Pre-Antibiotic	FMT dosage	FMT delivery route	#FMT	FMT state	Donor	Follow-up	Definition clinical remission	Definition clinical response	Endoscopic endpoints (index)	Unique clinical endpoints
Xiang et al[30]	Metoclopramide and esomeprazole prior to infusion (except for mid gut tubing delivery).	N	150-200ml (60cm^3 in 100 ml)	Endoscopy, nasojejunal tube and mid-gut TET except 1 patient who underwent colonic TET	Multiple: median 3.5 (IQR 2-5)	Fresh/ frozen	Healthy relatives or friends or China fmtBank (individual)	52 weeks	HBI ≤ 4	HBI >3, and remission	N	Improvement in therapeutic targets (pain, diarrhoea, hematochezia, fever, steroid-dependence, fistula).
Wei et al[29]	Bowel lavage (PEG).	Vancomycin 500mg oral twice a day for 3 days.	60g in 300ml	Nasojejunal tube	Single	Fresh	Healthy unrelated donor (individual)	4 weeks	CDAI score < 150 and CRP level < 10mg/L	Decrease of CDAI > 70	N	Quality of life score.

Vaughn et al[35]	Bowel lavage (magnesium citrate).	N	50g in 250ml	Endoscopic infusion (colonoscopy)	Single	Frozen	Healthy unrelated men (individual)	12 weeks	HBI < 5	Decrease of HBI > 3, without medications	N	Quality of life score.
Gutin et al[33]	Bowel lavage.	Rifaximin 550mg oral 3 times a day for 3 days (n=3)	250ml	Endoscopic infusion (colonoscopy)	Single	Frozen	OpenBiome stool bank (individual)	52 weeks	HBI < 3	Decrease of HBI ≥ 3	Y (SES-CD)	Difference in clinical parameters between responders and nonresponders.
Vermeire et al[36]	Bowel lavage (PEG).	N	200g in 400ml	Nasoduodenal tube or rectal tube	Multiple: 2 (1 day apart)	Fresh	Healthy relatives or friends (individual)	26 weeks	Not specified	Not specified	Y (SES-CD/CDEIS)	-
Karolewska-Bochenek et al[37]	Proton pump inhibitor and and ondansetron on the morning of the infusion.	N	50g in 50ml)	Endoscopic (gastroscopy) or a nasoduodenal tube	Multiple: 8 (within 12 days)	Frozen	Healthy unrelated donors (individual)	2.5-5 weeks	PCDAI ≤ 10	Decrease of PCDAI ≥ 15	N	-
Goyal et al[34]	Omeprazole (dose up to 20 mg twice daily) or equivalent starting 5 days before the procedure for 7 days. Loperamide	Metronidazole/vancomycin (max 500 mg/dose oral 3 times a	150g in 250-300ml	Endoscopic infusion (via gastroscopy and colonoscopy)	Single	Fresh	Healthy friends or immediate family or 1st degree relatives	26 weeks	PCDAI 0, and normalisation of biomarkers	Decrease of PCDAI ≥ 12.5	N	Difference in clinical parameters between responders and nonresponde

	2 hours prior to infusion.	day for 5 days)					(individual)					rs.
Suskind et al[32]	Omeprazole on the day before and morning of FMT. MiraLAX three times a day for 2 days prior to FMT.	Rifaximin 200 mg oral 3 times a day for 3 days	30g in 100-200ml	Nasogastric tube delivery (3-minute delivery, flushed 15ml of normal saline over 1 minute)	Single	Fresh	Parent of patient (individual)	12 weeks	PCDAI < 10	Not specified	N	-

TET: transendoscopic enteral tubing; HBI: Harvey-Bradshaw index; PEG: polyethylene glycol; CDAI: Crohn's disease activity index; CRP: C-reactive protein; SES-CD: simple endoscopic score for Crohn's disease; CDEIS: Crohn's disease endoscopic index of severity; PCDAI: paediatric Crohn's disease activity index; FMT: faecal microbiota transplantation.

Table 3. FMT protocols and endpoint definitions in randomised controlled trials

Study	Intervention	Comparison	Pre-FMT medications	Pre-Antibiotic	Frequency	Type of faeces	Donor	Follow-up	Clinical response	Clinical remission	Endoscopic endpoints	Unique clinical endpoints
Yang et al[23]	Gastroscopy group: 200g stool in 500ml. Colonoscopy group: 200g stool in 500ml.	N/A	Gastroscopy: omeprazole evening before and the morning of FMT, metoclopramide post-FMT. Colonoscopy: bowel lavage (macrogol) evening before, and loperamide prior to FMT.	N	Multiple: 2 (1 week apart)	Fresh	Healthy relatives, partners, or volunteers (individual)	8 weeks	CDAI < 150	Decrease of CDAI > 100	Y (SES-CD)	Difference between FMT via gastroscopy and colonoscopy infusions.
Sokol et al[24]	Colonoscopy: 50-100g stool in 300ml solution.	Physiological serum	Bowel lavage (PEG).	N	Once	Fresh	Healthy unrelated donors (individual)	24 weeks	Not specified	Not specified	Y (CDEIS)	Clinical flare: CDAI > 220 points, or between 150 and 220 with an increase > 70, or by the need for surgery or to start a new medical treatment for CD.

FMT: faecal microbiota transplantation; CDAI: Crohn's disease activity index; SES-CD: simple endoscopic score for Crohn's disease; PEG: polyethylene glycol; CDEIS: Crohn's disease endoscopic index of severity; CD: Crohn's disease.

Table 4. Clinical outcomes

Study	Early response (2 weeks)	Induction response (2-8 weeks)	Maintenance response (8-52 weeks)	Clinical remission	Endoscopic remission	Other
Xiang et al[30]	-	Single FMT month 1 131/174 (75.3%).	Final follow up 76/174 (43.7%). Multiple FMTs 64/109 (58.7%).	Single FMT 12/131 (9.2%). Multiple FMT 23/109 (21.1%). Final follow up 35/174 (20.1%).	-	Total therapeutic targets decreased: months 1, 3, 6, 12, 24 and 36 months ($P < 0.001$). 50% of steroid-dependent patients steroid-free 6 months after FMT (single FMT 8/18; multiple FMTs 1/18).
Wei et al[29]	-	-		-	-	No CD patients achieved an IBDQ total score >170 four weeks after FMT. Change in IBDQ score was ⩾50 in 2/3 (66.7%) of CD patients.
Yang et al[23]	Week 2 21/27 (77.8%).	-	-	Week 2 18/27 (66.7%). Week 8 9/27 (36%).	No patients endoscopic response or remission at week 8.	No significant differences were seen between the gastroscopy and colonoscopy groups.
Vaughn et al[35]	-	Week 4 11/19 (58%). Week 8 8/19 (42.1%).	Week 12 6/19 (31.6%).	Week 4 10/19 (53%).	No patients achieved endoscopic response at week 12.	Significant improvement in quality of life score.
Gutin et al[33]	-	Week 4 3/10 (30%).	-	Week 4 1/10 (10%).	No patients achieved endoscopic response at week 4.	Only significant difference between responders and non-responders was disease duration ($p=0.03$). There was no difference in clinical parameters including Harvey-Bradshaw Index, stool frequency, pain, C-reactive protein, and faecal calprotectin

						between responders and nonresponders.
Vermeire et al[36]	-	Week 8 0/6 (0%)	-	Week 8 0/6 (0%)	No patients achieved endoscopic response at week 8.	-
Sokol et al[24]	-	-	-	-	CDEIS decreased by week 6 (8.5 [4.6; 13.0] vs. 3.5 [1.0; 8.9]; p = 0.03) in FMT group.	Trend towards higher steroid-free clinical remission in FMT group. Steroid-free clinical remission: week 10 FMT 87.5% vs sham 44.4% and week 20 62.5% vs 33.3%.
Karolewska-Bochenek et al[37]	-	Weeks 2-4 2/2 (100%)	-	Weeks 2-4 2/2 (100%)	-	-
Goyal et al[34]	-	Week 4 5/7 (71%).	Month 6 3/7 (43%).	Month 6 2/7 (29%).	-	There was no difference in clinical parameters including age, disease duration, location, severity, or pretransplant medications between responders and nonresponders.
Suskind et al[32]	-	-	-	Week 2 7/9 (78%). Week 6 5/9 (56%). Week 12 5/9 (56%).	-	-

FMT: Faecal microbiota transplantation; CD: Crohn's disease; IBDQ: Inflammatory bowel disease questionnaire; CDAI: Crohn's disease endoscopic index of severity.

Table 5. Safety reporting

Study	FMT route	Serious ADRs	FMT related adverse events	Flare requiring medication switch or surgery
Cui et al[25]	Endoscopic infusion (midgut)	N	Fever: 2 (self-limiting). Diarrhoea: 7 (self-limiting). Both symptoms occurred 1-6 hours following FMT.	Incomplete information. Flare requiring additional therapy: at least 3 (immunomodulators or corticosteroids).
He et al[26]	Endoscopic infusion (distal duodenum for 23/25) and colon infusion (transendoscopic enteral tubing for 2 patients)	N	Small bowel obstruction: 2 within 1 month (perforation in 1 of these patients at 15 months). Fever: 2 within 6 hours and 3 days, respectively (1 required intravenous corticosteroids). Diarrhoea: 3 (self-limiting within 24 hours). Perianal pain: 1 patient with known perianal fistula (self-limiting).	Flare: 9 (all improved with subsequent FMTs). Flare requiring additional therapy: 2 (thalidomide; infliximab). Surgery: 3 (fistula resection; stoma creation).
Li et al[27]	Endoscopic infusion (distal duodenum via gastroscopy) or mid-gut/nasal-jejunal transendoscopic enteral tubing	N	Details not specified.	Incomplete information. Flare requiring additional therapy: at least 7 (immunomodulators or corticosteroids).
Wang et al[28]	Endoscopic infusion (distal duodenum via gastroscopy) or mid-gut/nasal-jejunal transendoscopic enteral tubing	N	Herpes zoster: 1. Bloating: 1. Vomiting: 1. Haematochezia: 1 (resolved with repeat FMT). Flatulence: 2. Abdominal pain: 4. Fever: 8 (this persisted in 1 patient requiring oral corticosteroids). Diarrhoea: 13 (this persisted in 1 patient requiring inpatient treatment with corticosteroids).	Incomplete information. Flare requiring additional therapy: at least 2 (corticosteroids).
Xiang et al[30]	Endoscopy, nasojejunal tube and mid-gut transendoscopic enteral tubing (except 1 colonic)	N	Details not specified.	Additional therapy: 52; Surgery: 42; Death: 4.
Zou et al[31]	Endoscopic infusion (midgut)	N	Fever: 3 (self-limiting). No other information specified.	Details not specified.
Wei et al[29]	Nasojejunal tube	N	Fever: 2 (self-limiting within 24 hours) - not specified whether these were CD/UC patients.	Details not specified.
Yang et al[23]	Endoscopic infusion (gastroscopy or colonoscopy)	N	Diarrhoea: 10. Abdominal pain: 5. Reflux: 4 (all in the gastroscopy group). Fever: 2. Nausea: 1. Belching: 2. Constipation: 1. Abdominal distension: 3. Most resolved	Details not specified.

			within 24 hours.	
Vaughn et al[35]	Endoscopic infusion (colonoscopy)	N	Systemic: 14. Infection: 1. Skin: 6 (including hives in 1 patient within 1 week of FMT requiring oral corticosteroids). Gastrointestinal: 77. Neurologic: 1. Respiratory: 4. Musculoskeletal: 6. Genitourinary: 1. Visual: 2.	Additional therapy: 7; Surgery: 1 (colectomy 8 weeks post-FMT).
Gutin et al[33]	Endoscopic infusion (colonoscopy)	N	Details not specified.	Additional therapy: 2. Flare without escalation in therapy: 1.
Vermeire et al[36]	Nasoduodenal tube or rectal tube	Y	Fever: 2 (requiring paracetamol). NOTE: Aspiration pneumonia occurred in a patient with UC requiring 14 days of broad-spectrum antibiotic therapy.	Details not specified.
Sokol et al[24]	Endoscopic infusion (colonoscopy)	N	Gastroenteritis: 1. Food poisoning: 1.	Flare: 9 (FMT: 3; Sham: 6).
Karolewska-Bochenek et al[37]	Endoscopic (gastroscopy) or a nasoduodenal tube	N	Vomiting: 2 (within 2 hours of FMT). Nausea: 1.	Flare: 0.
Goyal et al[34]	Endoscopic infusion (via gastroscopy into duodenum/jejunum and via colonoscopy into colon)	N	Adverse events in CD patients not separated from IBD cohort.	Flare: 3 (including 1 patient with clostridium difficile infection at week 13 that was treated with FMT).
Suskind et al[32]	Nasogastric tube delivery (3 minute delivery, flushed 15ml of normal saline over 1 minute)	N	Abdominal pain: 5. Abdominal bloating: 5. Diarrhoea: 4. Flatulence: 1. NGT related ADRs: 3. All symptoms improved within 48 hours of FMT.	Flare requiring additional therapy: 2 (infliximab; methotrexate and corticosteroids). 2 further patients had a flare after the 12-week follow-up.

N: No; FMT: faecal microbiota transplantation; CD: Crohn's disease; UC: ulcerative colitis; Y: yes; NGT: nasogastric tube; ADRs: adverse drug reports.

Figures

Figure 1. Flow Diagram

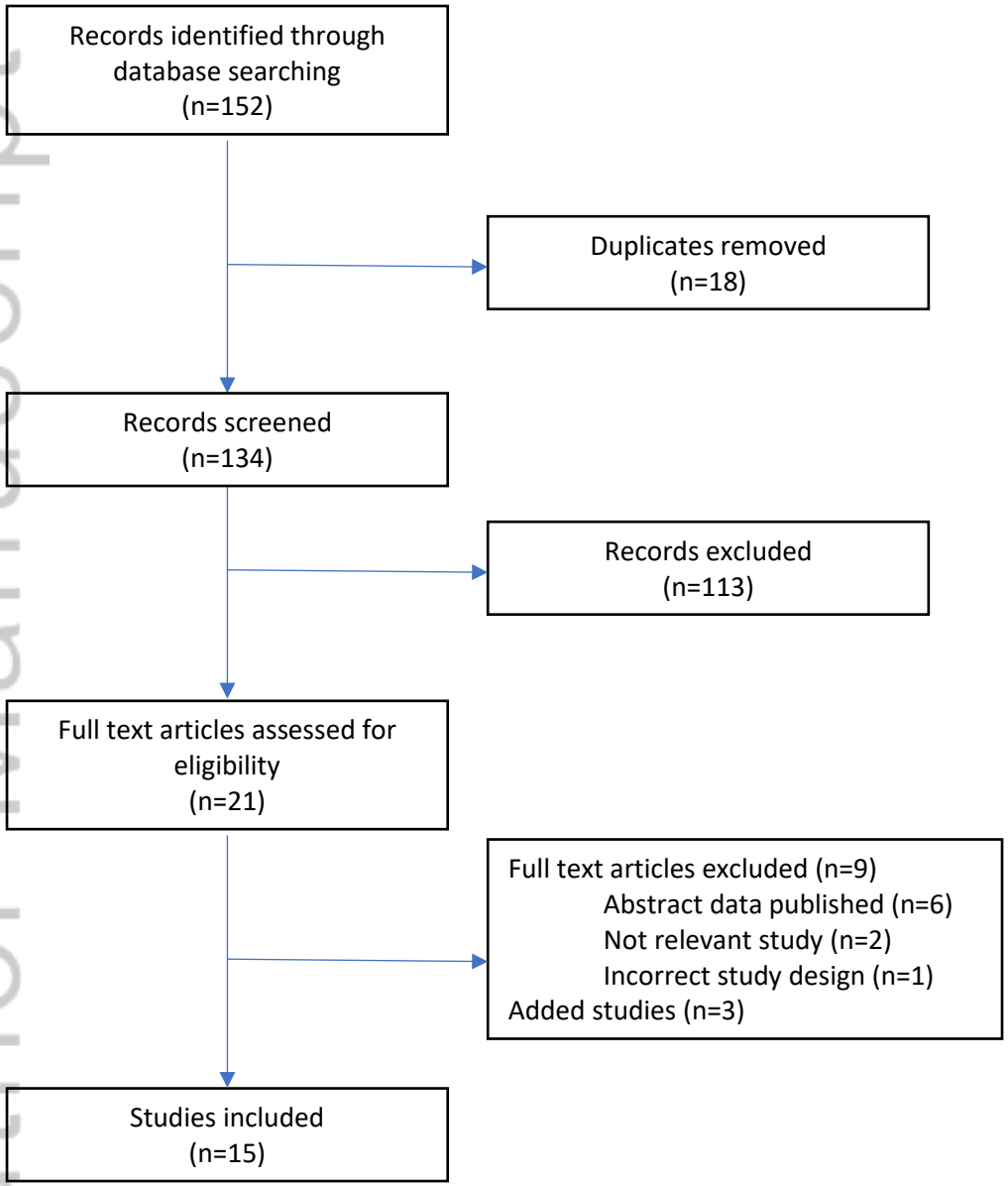

Figure 2. Quality Scores of Cohort Studies

STUDY	SELECTION	ASCERTAINMENT	CAUSALITY				REPORTING	TOTAL QUALITY SCORE	
			Alternative explanations	Challenge/ rechallenge	Dose-response	Follow up			
Cui et al[25]	+	+ +	-	-	-	+	+	5	Medium
He et al[26]	+	+ +	-	+	-	+	+	6	High
Li et al[27]	+	+ +	-	+	-	-	+	5	Medium
Wang et al[28]	+	+ +	-	+	-	+	+	6	High
Xiang et al[30]	+	+ +	-	+	-	+	+	6	High
Zou et al[31]	+	+ +	-	-	-	-	-	3	Low
Wei et al[29]	+	+ +	-	-	-	-	+	4	Medium
Vaughn et al[35]	+	+ +	-	-	-	+	+	5	Medium
Gutin et al[33]	+	+ +	-	-	-	+	+	5	Medium
Vermeire et al[36]	+	+ +	-	-	-	+	+	5	Medium
Karolewska-Bochenek et al[37]	+	+ +	-	-	-	-	+	4	Medium
Goyal et al[34]	+	+ +	-	-	-	+	+	5	Medium
Suskind et al[32]	+	+ +	-	-	-	+	+	5	Medium

+: each quality point assigned; -: no quality point assigned

Figure 3. Risk of Bias in Randomised Controlled Trials

STUDY	RANDOM SEQUENCE GENERATION	ALLOCATION CONCEALMENT	BLINDING PARTICIPANTS AND PERSONNEL	BLINDING OUTCOME ASSESSMENT	INCOMPLETE OUTCOME DATA	SELECTIVE REPORTING	OTHER BIASES	OVERALL JUDGEMENT	
Yang et al[23]	x	x	x	x	+	+	+	x	High
Sokol et al[24]	+	x	x	x	+	+	+	x	High

X: high risk of bias; +: low risk of bias

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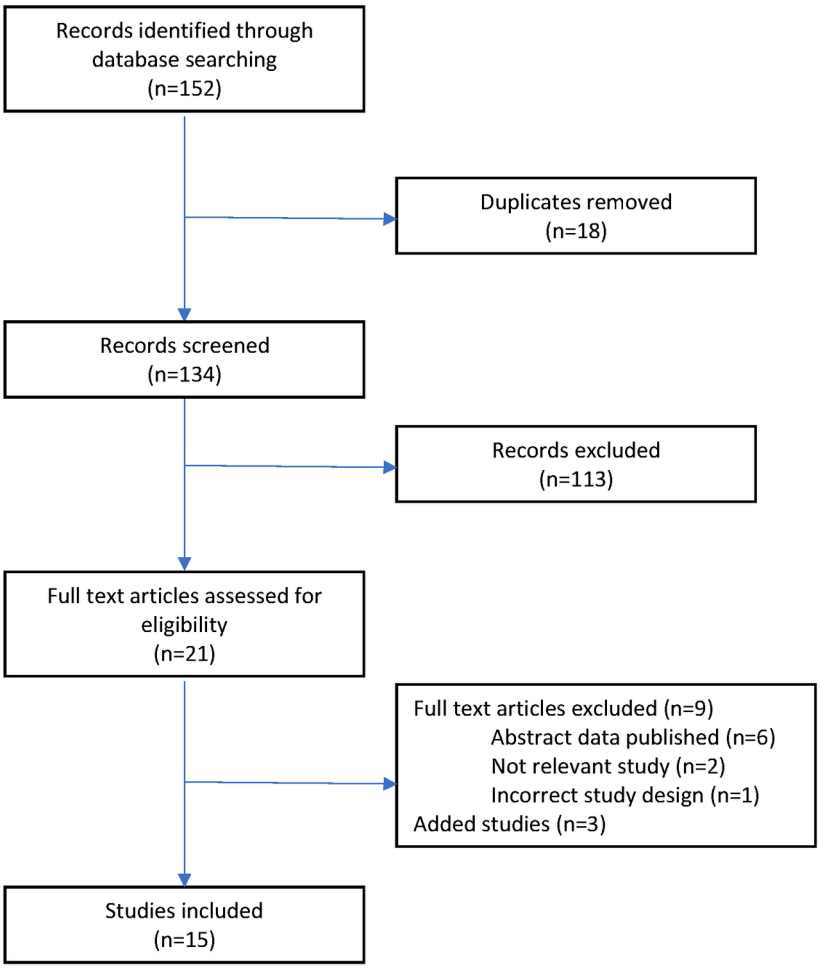

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Figure 2. Quality scores of cohort studies

STUDY	SELECTION	ASCERTAINMENT	CAUSALITY				REPORTING	TOTAL QUALITY SCORE	
			Alternative explanations	Challenge/ rechallenge	Dose-response	Follow up			
Cui et al[25]	+	+ +	-	-	-	+	+	5	Medium
He et al[26]	+	+ +	-	+	-	+	+	6	High
Li et al[27]	+	+ +	-	+	-	-	+	5	Medium
Wang et al[28]	+	+ +	-	+	-	+	+	6	High
Xiang et al[30]	+	+ +	-	+	-	+	+	6	High
Zou et al[31]	+	+ +	-	-	-	-	-	3	Low
Wei et al[29]	+	+ +	-	-	-	-	+	4	Medium
Vaughn et al[35]	+	+ +	-	-	-	+	+	5	Medium
Gutin et al[33]	+	+ +	-	-	-	+	+	5	Medium
Vermeire et al[36]	+	+ +	-	-	-	+	+	5	Medium
Karolewska-Bochenek et al[37]	+	+ +	-	-	-	-	+	4	Medium
Goyal et al[34]	+	+ +	-	-	-	+	+	5	Medium
Suskind et al[32]	+	+ +	-	-	-	+	+	5	Medium

+: each quality point assigned; -: no quality point assigned

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Figure 3. Risk of bias in randomised controlled trials

STUDY	RANDOM SEQUENCE GENERATION	ALLOCATION CONCEALMENT	BLINDING PARTICIPANTS AND PERSONNEL	BLINDING OUTCOME ASSESSMENT	INCOMPLETE OUTCOME DATA	SELECTIVE REPORTING	OTHER BIASES	OVERALL JUDGEMENT	
Yang et al[23]	x	x	x	x	+	+	+	x	High
Sokol et al[24]	+	x	x	x	+	+	+	x	High

X: high risk of bias; +: low risk of bias

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