ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΘΕΡΑΠΕΥΤΙΚΗ ΚΛΙΝΙΚΗ ΝΟΣ. ΑΛΕΞΑΝΔΡΑ

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ

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MSc: "Clinical Trials: Design and Conduct"

Διευθυντής

Ευάγγελος Τέρπος, Καθηγητής Ιατρικής Σχολής ΕΚΠΑ

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Όνομα: **ΜΑΡΙΑ ΜΕΓΑΠΑΝΟΥ**

Αρ. Μητρώου: **7450492200028**

Επάγγελμα: **Ιατρός**

Επιβλέπων καθηγητής ΜΔΕ: Μιχαήλ Λιόντος, Επίκουρος Καθηγητής, Ιατρική Σχολή ΕΚΠΑ

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ΑΘΗΝΑ 2024

Πρόλογος/ Ευχαριστίες

Οι ιδιοπαθείς φλεγμονώδεις νόσοι του εντέρου αποτελούν παθήσεις με προοδευτικά αυξανόμενο επιπολασμό. Καθώς αποτελούν χρόνια, μη ιάσιμα νοσήματα, η αντιμετώπισή τους επαφύεται σε ποικίλες φαρμακευτικές επιλογές, πολλές εκ των οποίων απαιτούν συχνές επισκέψεις σε δομές Υγείας είτε για την έγχυσή τους είτε για συχνές εργαστηριακές εξετάσεις.

Τα προβιοτικά κερδίζουν ολοένα και μεγαλύτερο έδαφος σε μια πληθώρα νοσημάτων που δεν αφορούν αποκλειστικά το γαστρεντερικό σωλήνα. Δεδομένης της ευρέας διαθεσιμότητάς τους, χαμηλού κόστους και το περιορισμένο προφίλ παρενεργειών, έχουν κεντρίσει το επιστημονικό ενδιαφέρον για το ρόλο τους στην επίτευξη ύφεσης και διατήρησης αυτής στις ΙΦΝΕ. Βάσει αυτών, η παρούσα διπλωματική εργασία αποσκοπεί στο να αποσαφηνίσει τη συμβολή των προβιοτικών στη διατήρηση της ύφεσης της νόσου Crohn.

Θα ήθελα να ευχαριστήσω τον υπεύθυνο Καθηγητή, κ. Λιόντο Μιχαήλ, για την πολύτιμη καθοδήγησή του. Επίσης, θα ήθελα να ευχαριστήσω την Κομνηνού Ελένη για την αμέριστη βοήθειά της. Τέλος, θα ήθελα να ευχαριστήσω την οικογένεια μου και τον Γιώργο για τη στήριξή τους καθ'όλη τη διάρκεια αυτών των μηνών. Θα είμαι για πάντα ευγνώμων.

Table of Contents

1. Abstracts

A. <u>Περίληψη</u>

ΣΚΟΠΟΣ

Οι ιδιοπαθείς φλεγμονώδεις νόσοι του εντέρου (ΙΦΝΕ), που απαρτίζονται κυρίως από τη νόσο του Crohn (NC) και την Ελκώδη Κολίτιδα (ΕΚ), είναι χρόνια νοσήματα που επηρεάζουν κυρίως το γαστρεντερικό σωλήνα. Ο ρόλος του ανθρώπινου εντερικού μικροβιώματος του ανθρώπινου εντέρου στις ΙΦΝΕ έχει εδραιωθεί καθώς τα δεδομένα καταδεικνύουν ότι και στις 2 νόσους, επηρεάζονται συγκεκριμένα φύλα ως προς τον αριθμό και την ποικιλία με κυριαρχία παθογόνων και απώλεια των προστατευτικών βακτηρίων. Τα συχνώς χρησιμοποιούμενα προ- και πρεβιοτικά βρίσκονται υπό μελέτη για το ρόλο τους στην πρόκληση και διατήρηση της ύφεσης των ΙΦΝΕ με πολλά υποσχόμενα αποτελέσματα. Βάσει αυτών, ο σκοπός αυτής της συστηματικής ανασκόπησης είναι η διερεύνηση του ρόλου των προβιοτικών στη διατήρηση της ύφεσης στους ασθενείς με έναρξη της νόσου Crohn σε ενήλικο ζωή.

ΜΕΘΟΔΟΙ

Η συστηματική αυτή ανασκόπηση διενεργήθηκε βάσει των οδηγιών του Cochrane Handbook για Συστηματικές Ανασκοπήσεις και τα αποτελέσματα καταγράφηκαν ακολουθώντας τις οδηγίες του PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyzes). Η αναζήτηση των μελετών έγινε στις βάσεις Cochrane και Pubmed: η ημερομηνία τελικής αναζήτησης ήταν η 31^{η} Δεκεμβρίου 2023.

ΑΠΟΤΕΛΕΣΜΑΤΑ

Η αναζήτηση απέδωσε 2015 συνολικά αποτελέσματα, εκ των οποίων 11 συμπεριλήφθησαν σε αυτή τη συστηματική ανασκόπηση. Τα ευρήματα σχετικά με τη χρήση των προβιοτικών, είτε ως μονοθεραπεία είτε ως επικουρική στις κλασικές θεραπείες, στη διατήρηση της ύφεσης της νόσου Crohn ήταν ατελέσφορα, με 4 μόνο μελέτες να καταδεικνύουν μια μη στατιστικώς σημαντική τάση προς τη χορήγηση των προβιοτικών για την πρόληψη της υποτροπής.

ΣΥΜΠΕΡΑΣΜΑΤΑ

Παρόλο που η συμβολή των προβιοτικών στη νόσο Crohn αμφισβητείται, αυτή η συστηματική ανασκόπηση επισημαίνει την ανάγκη για περαιτέρω έρευνα γύρω από την επίδραση των προβιοτικών στο μικροβίωμα των ασθενών με ΙΦΝΕ καθώς και περισσότερες τυχαιποιημένες μελέτες με μεγαλύτερα δείγματα ασθενών και προτυποιημένα θεραπευτικά σχήματα, με στόχο τη δημιουργία εξατομικευμένων θεραπειών με προβιοτικά που θα φέρουν αποτελέσματα στη νόσο Crohn.

B. Abstract

BACKGROUND

Inflammatory bowel diseases (IBD), consisting mainly of Crohn's disease (CD) and Ulcerative Colitis (UC), are chronic illnesses mainly affecting the gastrointestinal tract, but not only. The role of the human gut microbiome in IBD has been established as findings suggest that in CD and UC, certain phyla, though different in each disease, are affected in count and diversity, with dominance of pathogenic bacteria and loss of protective ones. The commonly used pro- and prebiotics are under investigation for their role in the induction and maintenance of remission of IBD, with promising results. Considering the above, the aim of this systematic review is to further investigate the role of probiotics in the maintenance of remission in adult-onset Crohn's Disease.

MATERIALS & METHODS

The systematic review was conducted according to the recommendations of the Cochrane Handbook for Systematic Reviews and the results were reported following the rules of the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA). Eligible studies were identified in the Cochrane database and PubMed; end of search date was the 31st of December 2023.

RESULTS

The search identified a total of 2015 items, of which 11 were included in this systematic review. The findings concerning the use of probiotics, either as monotherapy or as adjunctive therapy to traditional treatments, in the maintenance of remission of Crohn's disease were inconclusive, with 4 trials indicating a not statistically significant trend favoring their administration for relapse prevention.

CONCLUSIONS

Although the role of probiotics in Crohn's disease is doubted, this systematic review highlights the need for further research on the effect of probiotics on the microbiome profiles of IBD patients as well as large-scale, randomized controlled trials with standardized probiotic formulations, aiming at personalized probiotic therapies with valuable results in Crohn's disease.

2. Introduction

A. DEFINITIONS

Inflammatory bowel diseases (IBD), consisting mainly of Crohn's disease (CD) and Ulcerative Colitis (UC), are chronic illnesses mainly affecting the gastrointestinal tract but not only. They are characterised by a chronic course of recurrent inflammation in various parts of the gastrointestinal system. Their underlying cause, though unknown, is thought to be a combination of genetic predisposition, immunological and environmental factors[1],[2] .

Crohn's disease is characterized by a discontinuous pattern of transmural inflammation in every part of the gastrointestinal tract, with the proximal colon and ileum being mainly affected, suggesting that the affected sections are revolving with normal ones[3]. Transmural inflammation may lead to fibrosis, strictures and, therefore, intestinal obstruction as well as microperforations and fistula formation.

On the other hand, ulcerative colitis induces a continuous course of mucosal inflammation initiating from the rectum and ascending until the ileus [1,2]. Despite being confined to the mucosa, UC may be complicated with strictures as a result of recurrent episodes of inflammation. Finally, while both entities are correlated with increased risk for colorectal cancer (CRC), UC has been reported to have a greater risk of CRC than CD[4].

B. EPIDEMIOLOGY

It is estimated that 2.5 to 3 million people in Europe[5] and more than 4.90 million people worldwide suffer from IBD[6]. The prevalence of IBD is increasing and, therefore, posing a heavy health and socioeconomic burden. According to Global Burden Disease, the number of IBD cases had been raised by 47.45% in 2019 compared to 1990, with the highest increase being recorded in the newly industrialized regions in Asia and South America thus, conflicting with the previous theory of IBD prevalence following a "western" pattern [6,7]. According to Ng et al, several studies have suggested that the incidence of IBD in the Western world has been stable or even decreasing, although the incidence of pediatric-onset IBD continuously rising[8].

It seems that the incidence and prevalence rates of IBD in newly industrialized countries have been following the rates of western countries seen 40 years earlier[9]. As far as ethnicity is concerned, people of white and Jewish origin have a greater risk of developing IBD. Nowadays, an increasing incidence of IBD is being reported in Hispanic and Asian people with descendants of people who immigrated to regions with higher IBD prevalence being at greater risk of developing IBD[10].

The incidence rates of IBD are highest among the second to the fourth decade of life[9] while the diagnosis may occur at any age. It is noteworthy that the number of children less than 10 years of age diagnosed with IBD is increasing in certain western areas as well as the number of seniors suffering from IBD is growing following the earlier detection of the disease and the improvements in healthcare. Small but not statistically significant differences in the incidence of IBD between female and male patients have been noted with CD prevailing in females.

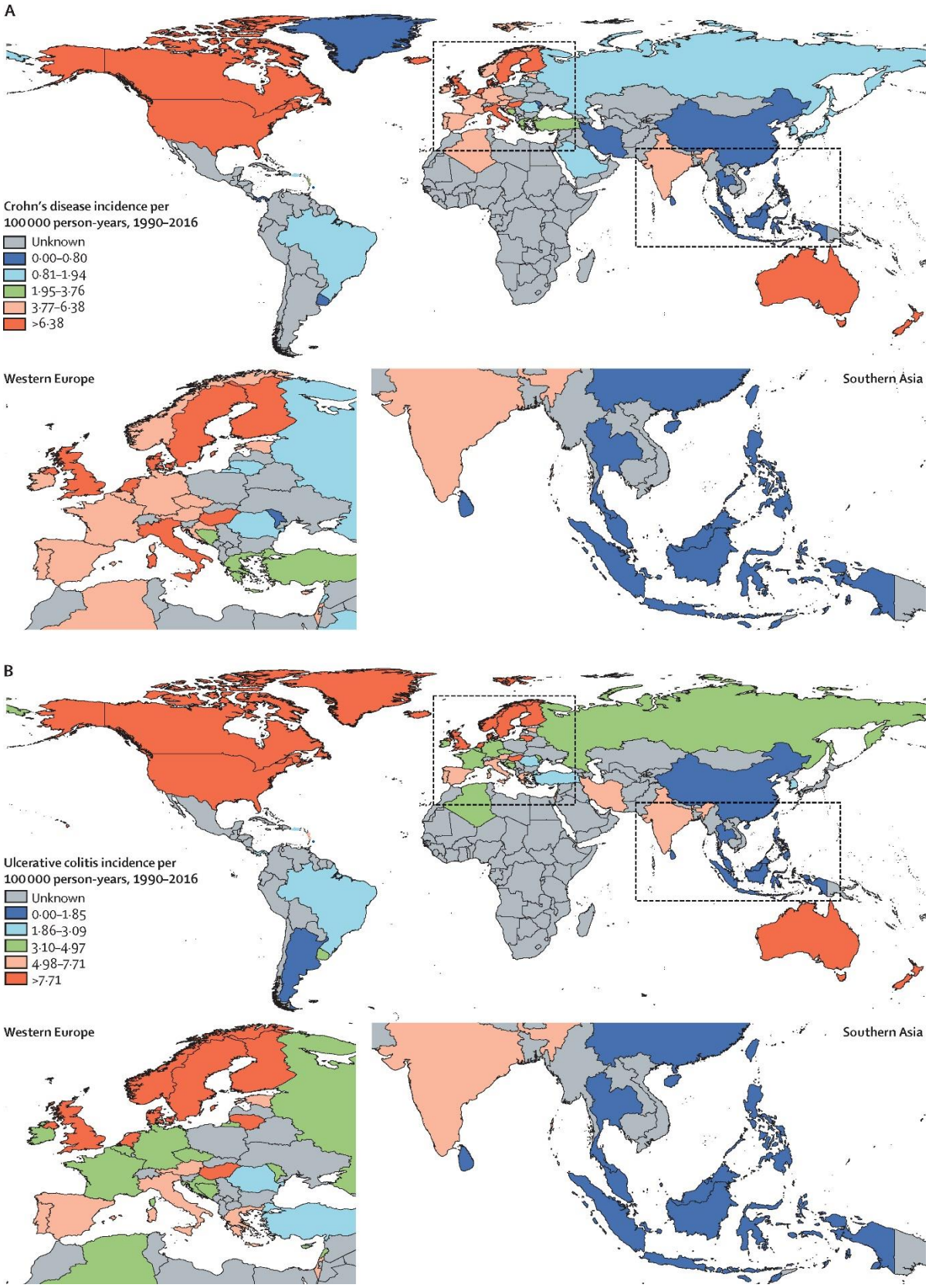

Figure 1: Map of worldwide incidence (1990-2016) in quintiles for (A) CD and (B) UC[11].

C. ETIOLOGY

To date, despite vigorous studying, the reason behind IBD presentation remains unknown. The etiology is regarded as multifactorial with the interaction of genetic predisposition and environmental factors. The pathogenesis is based on tissue inflammation, caused by an immune response against luminal bacterial antigens. In genetically predisposed humans, immune cells such as CD4, CD8 T-cells, B-cells and natural killers, infiltrate the mucosal barrier of the gastrointestinal tract producing a variety of inflammatory cytokines, such as interleukins, TNF-a and IFN-γ which further aggravate the intestinal inflammation[12] .

The genetic background for IBD refers to a variety of genes -approximately 200- which induce pathways determining susceptibility, disease specificity and phenotype. The genes coding the nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and autophagy-related 16 like 1 (ATG16L1) were among the first to be associated with IBD as their defects impair antibacterial response and cause a dysregulation in innate and adaptive immune response[2,13]. Notably, many genes involved in IBD are also met in other immune disorders, such as ankylosing spondylitis and psoriasis.

The role of the human gut microbiome in IBD has been vigorously studied during the last decades. The GI microbiome is abundantly diverse with more than 1100 species[14]. Depending on the long-term diet followed, each gut microbiome can be categorized in 3 distinct types, based on the predominant microbe- Type 1 for *Bacteroides*, type 2 for *Prevotella* and type 3 for *Ruminococcus*[15]. Findings suggest that in CD and UC, certain phyla, though different in each disease, are affected in count and diversity, with dominance of pathogenic bacteria and loss of protective ones. These alterations are characterized with the term "*dysbiosis*"[16].

According to the Theory of Endobiogeny, *dysbiosis* is defined as an imbalance in amount, variety, and/or location of microbial organisms, be they commensal, noncommensal, and/or pathogenic[17]. It is well documented that in IBD the phyla *Firmicutes* is decreased both in count and diversity, while in a considerable number of studies, the phylum *Bacteroidetes* appears to be increased. These two account for 90% of the phylogenetic categories in a healthy gut. On the other hand, the phylum *Proteobacteria*, in which *E. Coli* belongs, include most pathogenic bacteria linked with IBD. This disruption in the anaerobic microbes is thought to cause an increase in the amount of oxygen needed in the intestine microorganisms.

The intestine is contiguous to a variety of different microbes leading to a constant and balanced interaction between the epithelium and the microorganisms. This interaction is primarily affected in patients with IBD. The normal colonic epithelium expresses a diverse amount of Pattern Recognition Receptors (PRR) which identify any possible pathogens. A disruption in the expression and function of certain PRRs (toll-like receptors, NOD2) has been studied in IBD. Any impairment of these PRRs enables the translocation of bacteria to the lamina propria. An increased inflammatory cytokine response with concomitant down-regulation of "protective" cytokines has also been observed. Until now, there is no evidence on which phenomenon precedes[14].

Environmental factors that have been linked to the development of IBD include the hygiene hypothesis, smoking, diet, early antibiotic use and breast feeding. The hygiene hypothesis lies on the assumption that low exposure to microbes in childhood is related with defects in the adaptive immune system which predisposes the child to a pathologic immune response after exposure to certain pathoges[18]. It is considered that GI infections dispose a higher risk for the occurrence of IBD with the literature being considerably

heterogenous[19] . According to Shaw et al., patients with IBD diagnosed in childhood are more likely to have a history of antibiotic use in their first year of life.

Breast milk contains a variety of microbes that influence the infant's intestinal microbiome and epithelium. Microbiota in breast milk promote immune tolerance, prevent infection, and play a role in the maintenance of the epithelial barrier through an immune-mediated influence on intestinal microbiota composition. More specifically, the oligosaccharides in breast milk act as prebiotics for the child as they prevent the adhesion of enteropathogenic E. Coli, V.cholerae and Salmonella fyris to the epithelial cells.

Lifestyle changes have, also, an impact on the development of IBD. High consumption of total fats and meat were linked to higher rates of IBD fact which can be explained with the microbiome alterations of decreasing numbers of "protective" microorganisms and increasing of "harmful" ones. Regarding smoking, literature is inconclusive concerning its either protective or inimical role. However, there are certainly differences in the gut microbiota of smokers and non-smokers.

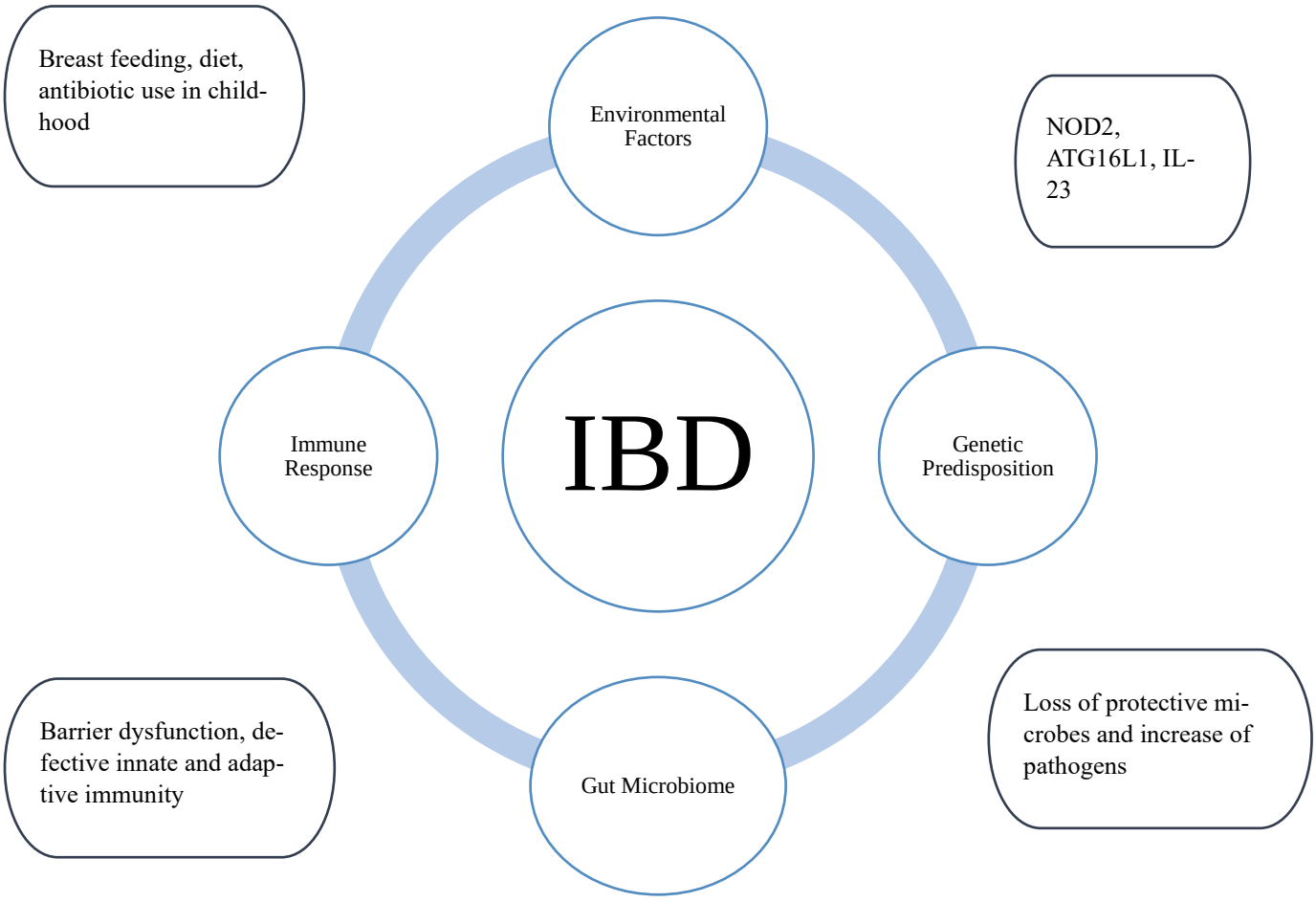

Figure 2: Etiology of IBD: The etiology of IBD is considered to be a compilation of environmental factors affecting a genetically predisposed gastrointestinal tract, thus generating a pathological immune response.

D. Clinical Presentation

The presenting symptoms of CD and UC differ due to various factors such as the site of the GI tract affected, the intensity of inflammation and the existence of extraintestinal complications. Up to 25% of IBD patients, especially with CD, delay their diagnosis due to mild clinical presentation thus increasing the risk of IBD-related surgery[20].

- Crohn's disease

Inflammation in CD may be located both in the mucosa and submucosa. The disease is distributed in a discontinuous, uneven pattern resulting in alternations of normal mucosa and inflammatory lesions-findings present in endoscopy. Histological hallmarks include focally enhanced inflammation, granulomas which are found in 15 to 60% of patients and crypt abscesses while aphthae are usually noted in the earlier course of CD. Later, multiform ulcerations which create a cobblestone appearance are indicative of CD[21].

The location of CD tends to be stable but may occasionally extend. Uncommon disease locations include the upper GI tract, with dysphagia and odynophagia in the esophageal disease and the appearance of peptic ulcer disease in the stomach and duodenum. Patients with disease in proximal small intestine are often younger and will eventually develop disease distally.

Although a proportion of patients maintain indolent behaviour, almost 70% of patients will suffer from the formation of fistula, stricture and subsequent intestinal obstruction and 60% of patients will require surgical intervention during the first 20 years since their diagnosis [22]. Constituting a tract between two epithelium-lined surfaces, fistulas, mainly perianal, represent the most common complication of CD occurring in 15% to 50% of patients.

- Ulcerative Colitis

Unlike CD, UC induces only mucosal inflammation-in the rare occasion of fulminant colitis, transmural inflammation may be noted. The disease progresses continuously, beginning from the rectum and ascending in the large intestine. Approximately 45% of patients have limited to the rectosigmoid disease, 35% present with colitis in the sigmoid and descending colon ("left-sided colitis") and 20% have pancolitis, in which ileitis may be also present, though infrequently.

Histologically, ulcerations and crypt abscesses are the main characteristics of UC. Polypoid protrusive normal mucosa among inflammatory lesions creates the illusion of pseudopolyps, which are also usually observed. Strictures and fistulas are rarely met.

Regarding the presenting symptoms, diarrhea is the most common complaint in both UC and CD. The nature of diarrhea may include intermittent or persistent courses, large or small as well as with or without bloody commixture, with the latter being most prevalent in UC. The causes vary among inflamed mucosa, fistulas, and bile salt malabsorption.

Abdominal pain is a common manifestation of CD and may result from inflammation, fistulas, abscess, or obstruction in the ileum or colon, while reported to be continuous, intermittent, or colicky. The ileal inflammation may lead to fibrotic stenosis inducing a longstanding colicky pain, nausea and vomiting.

Additionally, patients may report anorexia, weight loss and frequent bowel movements. Extraintestinal manifestations may affect the hepatobiliary, pulmonary, myoskeletal, immune, renal and ocular system.

E. Diagnosis, Classification and Evaluation of Disease Activity

The approach towards the establishment of an IBD diagnosis consists of 4 elements: clinical evaluation, endoscopic, histologic and serologic studies. Although endoscopy with biopsies is the gold standard, serologic markers (ASCA, ANCA, CRP, ESR), radiologic imaging modalities (CT scan, Magnetic Resonance Enterography), and small bowel capsule endoscopy (SBCE) prove to be useful tools.

Considering the variety of the phenotypical behavior of CD, the Montreal Classification has been incorporated into clinical and research practice. Succeeding the Vienna Classification in 2008, Montreal Classification may be used for the classification not only of CD but also of UC and indeterminate colitis[23].

Age at diagnosis (years)	A1	<16
	A2	17-40
	A3	>40
Location	L1	Ileal
	L2	Colonic
	L3	Ileocolonic
	L4	Isolated upper GI
Behaviour	B1	Non-stricturing, non-penetrating
	B2	Stricturing
	B3	Penetrating
	p	Perianal disease

Table 1: The Montreal Classification System. L4 is added to L1-3 in case of concomitant upper GI disase while p is added in concomitant perianal disease. Adapted from Satsangi, Silverberg, Vermeire, et al.

The activity of CD may be measured using a variety of modalities. Apart from the self- and physical assessment, disease activity can be estimated through endoscopic procedures and laboratory tests, such as CRP, ESR and fecal calprotectin.

1. Crohn's Disease Activity and Harvey-Bradshaw Indexes

In clinical trials, the Crohn's Disease Activity Index (CDAI) is the most prominent. Developed in 1976 by Best et al[24], it is the gold standard in the assessment of CD in clinical trials considering 8 parameters, with the 3 of them coming from a 1-week patient-reported diary (Table 1). Ranging between 0 and 600, the activity index may be classified into 4 categories:

- **Clinical remission**: With a score less than 150, the patient is asymptomatic without inflammatory implications.
- **Mildly active CD**: Scoring between 150 and 219, the patient can tolerate oral food intake, without presenting dehydration, abdominal pain, obstruction or weight loss of more than 10%.

- **Moderately active CD**: With a score 220 to 450, the patient is unresponsive to the treatment given to the mild CD while exhibiting anemia and symptoms suggestive of systemic inflammation such as fever, tachycardia, abdominal mass, nausea and weight loss.
- **Severe to fulminant disease**: A patient with a score of 450 or higher has deteriorating symptoms of high fever, intestinal obstruction, weight loss and abscess formation, despite receiving steroids or biologic agents.

Variable		Weight
General wellbeing *Summary of 7-day ratings*	Generally well =0 Slightly under=1 Poor=2 Very poor= 3 Terrible= 4	7
Number of liquid stools *Summary of 7 days*		2
Abdominal pain *Summary of 7-day ratings*	None= 0 Mild= 1 Moderate= 2 Severe= 3	5
Abdominal mass *Summary of 7 days*	No= 0 Questionable= 2 Definite=5	10
Antidiarrheal Agents *Use during the last week*	No= 0 Yes= 1	30
Extraintestinal complications *Arthritis/arthralgia, iritis/uveitis, Erythema nodosum, Pyoderma gangrenosum, Aphthous stomatitis, Anal fissure/fistula/abscess, Fever >37.8°C*		20
Hematocrit *(Expected–observed Hct)*	Males: 47-observed Females: 42-observed	6
Body weight	[1 − (body weight/standard weight)] × 100	1

Table 2: CDAI score. To calculate the CDAI, the scale is multiplied by the weighting factor for each variable, and then all 8 weighted variables are added.

Correlating with the CDAI[25], the Harvey-Bradshaw Index (HBI) of Crohn disease activity in adults evaluates predominantly the patient's symptoms (i.e. general well-being, liquid stools, abdominal pain and mass) and the existence of extraintestinal manifestations, fluctuating between 0 and 100 points[26].

Of note, any reduction of 100 points or more in CDAI and 3 points of more in HBI responds to clinical response or, otherwise, a clinically significant improvement. The value of these scoring systems is limited by the subjectivity of the symptoms included, the "interobserver" variability, the inaccuracy in patients with previous surgical management as well as fistulizing and stenotic forms of disease.

2. Endoscopic Modalities and Indexes

As previously mentioned, endoscopy (ileocolonoscopy/upper GI endoscopy) with the collection of biopsies which will set the diagnosis, is the definitive modality for the visualization of the GI tract and. The endoscopist will face an inflamed mucosa with ulcers, aphthous erosions and luminal contraction, well-demarcated regions, obstructive and fistulizing areas.

Besides CDAI, other composite scoring systems, such as the International Organization of IBD, the De Dombal's index, the St Marks Crohn Index and the Talstad Index, are also being used although less frequently. Since each of these is a combination of subjective and objective features, the need for a more objective assessment has emerged. It has been observed that the symptoms of IBD do not always correlate with the activity of the disease as seen in endoscopy, radiologic imaging (CT, MRI) and laboratory values (ESR, CRP, fecal calprotectin). Mucosal healing seen in endoscopy has become one of the principal targets of the treatment posing, therefore, the need to develop scores which correspond to this necessity. Crohn's Disease Endoscopic Index of Severity (CDEIS) and Endoscopic Crohn's Disease Index (SES-CD) are the two widely used tools for the endoscopic assessment of CD.

Being the first endoscopic evaluation score, CDEIS is a complex system which evaluates the intestinal extent of CD and the extent of ulcerated surface as well as the presence of deep and superficial ulcers, by dividing the large intestine into 5 segments[27]. A score of more than 5 corresponds to active disease. Because of its complexity for routine practice despite being quite reliable, the Endoscopic Crohn's Disease Index (SES-CD) is a simplified form of CDEIS which examines the extent of surface affected by ulcers or CD, the presence and size of ulcerations and the presence and type of stenoses. CDEIS and SES-CD are correlated as seen by the equation [28]:

$$\text{CDEIS}=0.76*\text{SES-CD}+0.29$$

Their advantages rely on their sensitivity for endoscopic variability, the freedom for comparison of different endoscopic methods and their prognostic ability. Notably, the corticosteroid-free clinical remission can be predicted through this score, as demonstrated by Ferrante M, Colombel JF, Sandborn WJ, et al[29]. The presence of deep and extensive ulcerations is associated with a more aggressive pattern of disease and higher chances of surgical management[30] . On the other hand, both scores are criticized for their complexity and the need for post-procedure time to be scored.

According to ECCO consensus, the recurrence of CD after surgical management with ileo-cecal anastomosis should be assessed with the Rutgeerts' score. In this index, the anastomotic site or the afferent ileal loop is appraised for the existence of apthous lesions, ulcers, ileitis, cobblestone pattern and strictures. It has been demonstrated that patients with a grading of i2 or more present a more aggressive in clinical and surgical terms course of disease[28].

Index	Variable	Site of Applicability
CDEIS	Superficial/deep ulcers, surface affected by ulcers, surface affected by disease, non-/ ulcerated stenosis	Large Intestine
SES-CD	Ulcer size, area size of ulcers, extent of disease, type of intestinal stenosis	Large Intestine
Rutgeert's score	Aphtous ulcers, ulcers, aphtoid ileitis, erythema, cobblestone, stenosis	Anastomotic site or the afferent ileal loop in ileocecal anastomosis

Table 3: Endoscopic scores for the assessment of Crohn's disease. Adapted from ECCO Consensus for endoscopy in inflammatory bowel disease[28]

The necessity for direct visualization of the small bowel which could not be completed with conventional endoscopy, led to the development of small bowel endoscopic techniques. These endoluminal imaging modalities include push enteroscopy, small bowel capsule endoscopy (SBCE) and balloon-assisted endoscopy. According to ECCO guidelines, SBCE may be used for the initial diagnosis of CD in case a definitive diagnosis of CD cannot be established and the patient does not present with obstructive features. SBCE should be reserved only for the differential diagnosis of iron-deficiency anemia or the further investigation of unexplained symptoms-not for the evaluation of disease activity[2,28,31].

3. Biomarkers

Although the diagnosis and assessment of CD activity rely primarily on endoscopic and radiologic techniques, certain biomarkers are used in everyday practice to assist the monitoring of CD. Low-cost, absence of intervention and wide accessibility are the advantages of the use of serologic and fecal biomarkers, despite the contradictive results in scientific research for their use as markers of CD activity[31]. The most frequently used biomarkers are c-reactive protein (CRP) and fecal neutrophil-derived.

C-reactive protein (CRP) is a nonspecific, acute phase protein with a short half-life, derived from the liver in cases of inflammation and stimulated by interleukin-6 (IL-6), initially, and then, by tumor necrosis factor-a (TNF-a), and 1β (IL-1β). High levels of CRP are used to assist the differential diagnosis of IBD[32]. Despite being a nonspecific marker, its levels are correlated with disease activity. As Chamouard et al proved, low levels of CRP are predictive of low or absent CD activity, without establishing a certain cut-off for low activity versus inactive CD[33]. Additionally, the higher CRP levels are, the more increased surgery risk is[34]. The use of high sensitivity CRP (hs-CRP) has been studied as a more accurate tool for the association with CD activity. Hs-CRP increases proportionally with disease severity, especially in populations with elevated hs-CRP at diagnosis while it has been demonstrated that increased values of hs-CRP at diagnosis correlate with colonic disease location and stenotic/penetrating disease phenotype[35].
However, it should be noted that almost one third of patients with CD present with normal levels of CRP[36].

Fecal calprotectin (FC) is a calcium- and zinc- binding protein mainly derived from neutrophils and to a lesser extent, from monocytes. As intestinal inflammation attracts neutrophils, fecal calprotectin resembles the inflammatory process, thus making it a sensitive biomarker for it. It has proven correlation with disease activity and can be facilitated in CD diagnosis, monitoring of activity and treatment as well as recurrence

after surgical intervention[37]. With a cut-off of 50 μg/g of faeces, fecal calprotectin distinguishes among inactive, mildly-moderately and severely active CD, highly correlating with the endoscopic indexes SES-CD and CDAI comparing to other serologic markers, such as CRP[38]. Demonstrated by F.G.C. Penna et al, the combination of CRP and fecal calprotectin seems to increase the specificity of CD activity while the sole use of CRP and FC enhances the sensitivity. Therefore, their common use might assist the diagnosis of the CD phenotype when evaluated with the patient's clinical and endoscopic characteristics and prove to be more valuable for the clinician.

While fecal calprotectin is widely used, fecal lactoferrin (LF) constitutes a safe alternative for the diagnosis of IBD[39]. Being an iron binding glycoprotein with antibacterial effects, lactoferrin is secreted from the granules of neutrophils comprising a proportionate measure of the immigration of neutrophils to areas of inflammation and their excretion in the feces. It has been proven that LF has high specificity and sensitivity for the diagnosis and therapy monitoring of IBD with higher rates being observed in UC rather than CD[39,40]. Of note, both FC and LF may be detected through ELISA in the stools for the maximum of 7 days in different, however, temperatures: FC transcends as it can be observed in the feces at room temperature, thus making it easier-to-use.

4. Imaging Techniques

Imaging modalities, such as CT and MRI enterography as well as abdominal ultrasound (US), are a useful tool in establishing the extent of CD and the existence of stricturing or fistulizing disease.

The widely available Computed Tomography (CT) cannot fully examine the mucosal changes of CD. Nevertheless, CT enterography (CTE) may provide insight into mural and transmural disease active of the small and large intestine. The segmental enhancement of the small intestinal wall (*mural enhancement*), bowel wall thickening (>3 mm), increased attenuation of the peri-enteric fat and segmental dilatation of the vasa recta (*comb sign*) are the usual CD findings[2,31]. The sensitivity and specificity rates of CTE vary between 81% and 88%[41]. The wide use of CTE is limited by the exposure to radiation, the need for intestinal distension with oral and rectal contrast medicine and the use of intravenous contrast medication.

Magnetic Resonance Imaging (MRI) is an emerging alternative to CT. Despite not being generally accessible, MR enterography presents similar rates of specificity, sensitivity and diagnostic accuracy to CTE but surpasses CTE in the detection of strictures (0.95 vs 0.91, p:0.04)[41]. Its advantages are summarized in the ability to evaluate with high soft tissue contrast and in static and dynamic way mucosal and mural characteristics together with extraintestinal manifestations-all without exposure to radiation. Moreover, scoring systems which correlate MRE features, such as wall thickness, mural edema, contrast enhancement and ulcerations, with CD activity have been developed to increase the diagnostic accuracy of MRE. However, the lack of MRI installations, the need for pre-test preparation with oral, intravenous and possibly rectal contrast and the longer duration of the examination discourage the clinicians from broader application in the clinical practice.

Transabdominal ultrasound (US) is a noninvasive, widely available technique which can be used to assess the terminal ileum and the colon segmentally for the existence of wall thickness, stratification, stiffness and strictures. Apart from intestinal disease, US can be applied to the diagnosis of stenosis, extramural

manifestations such as fistulas and abscesses as well as the follow-up of CD[42]. Contrast-enhanced doppler US (CEUS), which allows the investigation of the vascularization pattern of CD, correlates with disease activity and may prove to be useful for the evaluation of response to treatment[43]. Nonetheless, the limited estimation of activity in the intestine in its full extent, intra-observer variability and the effect of bowel peristalsis and fat accumulation in the assessment of the intestinal wall restrict the application of US in CD.

F. TREATMENT

The therapeutic options for the treatment of CD differ according to the severity and location of the disease as well as the target imposed• induction or maintenance of remission. The 4 predominant categories of medications for the treatment of CD are summarized in Table 4.

Class		Frequently used medicines
5-Aminosalicylates		Mesalamine, Sulfasalazine
Glucocorticoids		Budenoside, Prednisone
Immunosuppressants	Thiopurines	Azathioprine (AZA), 6-Mercaptopurine (6-MP)
	Anti-Metabolite	Methotrexate
Biologic Agents	Anti-TNF	Infliximab, Adalimumab, Certolizumab pegol
	Anti-Interleukin Antibody	Ustekinumab, Risankinumab
	Anti-Integrin Antibody	Vedolizumab, Natalizumab
	JAK inhibitors	Upadacitinib

Table 4: Treatment choices for Crohn's disease.

For the induction of remission in mild CD, corticosteroids are used as a first-line therapy: budesonide is used for disease limited to the ileocolonic region and prednisone in case of pancolitis/left colitis. If the patient is responding to the treatment, gradual tapering is initiated aiming at the discontinuation and endoscopic follow-up every 6 to 12 months. However, in the scenario of CD flare, glucocorticoids are re-initiated with the addition of thiopurines.

In moderate to severe CD, the choice of agents depends on the disease characteristics (fistulizing verus obstructive pattern), prior treatment regimens, patient's characteristics and preferences. The induction of remission is attempted with the combination of an immunomodulator and an anti-TNF agent. Alternatively, anti-TNF monotherapy may be considered in patients over 60 years of age, young males, history of EBV infection or those with high infectious or malignant risk. Ustekinumab, an anti-Interleukin antibody agent, can be used either as a first-line monotherapy in patients with no prior exposure to biologic medicines or as a combination therapy with immunosuppressants. Finally, drugs classified as anti-integrin antibody may aid the induction of remission either alone or with anti-TNF agents.

The decision of medications for the maintenance of remission lies on the agent which induced the remission together with the patient's preferences. In the scenario of combination therapy, the anti-TNF medicine is continued until the patient presents with adverse events or flare of CD. Evidence is unclear towards the optimal timing for the discontinuation of thiopurines- usually are discontinued after 12-24 months from their initiation. Concerning the rest of biologic medications, long-term monotherapy is preferred for the

maintenance of remission. The patients who have achieved remission with corticosteroids or have deteriorated using biologic factors, thiopurines may pose a therapeutic option.

Surgery is indicated in cases of refractory to medical treatment disease as well as in the occurrence of complications such as obstruction, fistulas, abscesses and toxic colitis. Surgical intervention is also necessary in cases of dysplasia and uncontrollable gastrointestinal bleeding[44]. Even though approximately 70% of CD patients will undergo surgery at some point during their disease and unlike UC where surgery (i.e. proctocolectomy) has curative role, surgery was thought to achieve a rather long postsurgical period of remission[45]. However, recent advances in research have proved the postsurgical development of pathological lesions in otherwise healthy segments of the gastrointestinal tract, what has been described as "postoperative recurrence (POR)". The development is diagnosed initially histologically, then endoscopically, through direct vision via GI endoscopy, and then clinically, when the clinical features are eventually demonstrable[46]. Within 1 year postoperatively, 70 to 90% of patients will demonstrate endoscopic recurrence despite not experiencing symptoms suggestive of it. Evidence of clinical deterioration may be met in 30% of patients 3 years postoperatively and 60% 10 years afterwards [47,48]. Fortunately, the introduction of biologic therapies in the treatment of IBD has led to the reduction of surgical operations and recurrence for CD. Previous history of ileocolonic resection, smoking, penetrating behaviour and perianal disease constitute independent risk factors for surgical recurrence. On the contrary, postoperative use of biologic agents has been proved to be protective of recurrence[48].

Nowadays, pro- and prebiotics are commonly used for the preservation of the gut microbiota's homeostasis. ***Probiotics*** are live microorganisms which, when orally ingested in sufficient amounts, sustain a positive impact on the gastrointestinal tract through beneficial to the host immunomodulation and counteraction of pathologic bacteria. The majority of them are lactic acid producing bacteria (LAB) which induce the production of antibacterial components, prevent the binding sites of epithelial cells and assist the degradation of harmful bacteria. Some of the commonly used probiotics belong to the genera Escherichia coli Nissle 1917 (EcN), Lactobacillus, Bifidobacterium, Saccharomyces boulardii, Enterococcus and Streptococcus and can be found in yoghurt and other milk fermented products. Their safety profile, although not explored in-depth, includes mainly gastrointestinal symptoms, such as bloating and abdominal pain, whereas there are some reports about bacteremia due to intestinal permeability[49,50].

Firstly named by Gibson and Roberfroid, ***prebiotics*** constitute non-digestible substrate food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health[51]. Found in fruits and vegetables, prebiotics are usually saccharides and less commonly polyphenols and polyunsaturated fatty acids while they can be administered not only orally but also directly to other colonized areas of the human body[52]. Prebiotics should not be absorbed in the upper gastrointestinal tract and be "immune" to the process by enzymes. The purpose of their use lies on the stimulation of qualitative and quantitative growth of LAB, inhibition of pathogens through maintenance of the luminal pH in low levels, acceleration of mucosal healing and increase in the absorption of calcium, magnesium and iron. The combination of probiotics and prebiotics results in the creation of a ***Synbiotic***, which combines the advantages of both**.**

Their role in the induction and maintenance of remission of IBD is still under investigation, thus restricting their use as an adjuvant factor for remission in IBD. Several clinical trials have reported contradictory results[53]. The most encouraging results have been observed in patients with UC rather than CD[54,55]. However,

most clinical trials include a small number of participants and are characterized by heterogeneity[55]. Until now, the type, the dose and frequency of administration of probiotics in IBD has not been elucidated, thus posing an interesting topic for research.

Considering the above, the aim of this systematic review is to further investigate the role of probiotics in inducing and maintaining the remission of IBD and specifically Crohn's Disease in adults.

3. Methods

Search algorithm and eligibility criteria

The systematic review was conducted according to the recommendations of the Cochrane Handbook for Systematic Reviews. The results were reported following the rules of the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA).

The search was focused on human studies regarding the effect of probiotics in the progression of IBD and more specifically, Crohn's disease in patients to whom the disease was first presented in adulthood. Randomized controlled trials (RCTs), cohort, case-control and cross-sectional studies were considered acceptable. Eligible articles were determined by a search in the Cochrane database and PubMed for the period up to the 31st of December 2023. In the scenario of overlapping studies, the larger and latest study were chosen.

The following search algorithm was implemented: ("inflammatory bowel disease" OR "IBD" OR "Crohn's disease" OR "Crohn's" OR "Crohns disease" OR "ulcerative colitis" OR "UC") AND (probiotics OR "pro biotics" OR prebiotics OR "pre biotics" OR synbiotics OR "syn biotics"). The publication language was restricted to English and Greek. The references cited in the eligible articles were investigated in a "snowball" procedure to identify further studies eligible for our review. The titles and abstracts emerged from our search were evaluated by two independent authors (EK and MM) who worked independently. In case of disagreement, a third author (ML) assisted in the resolution. All studies which examined the efficacy of the administration of a probiotic or a symbiotic to participants with Crohn's disease in achieving or maintaining remission, either primarily or secondarily, were considered eligible.

Data collection and risk of bias assessment

The data obtained from eligible studies included: first author, year of publication, country of origin, study design, study period, sample size, number of subjects with IBD, number of subjects with Crohn's disease, number of subjects with ulcerative colitis, mean age, age range, selection criteria for the study population, disease activity score at the beginning of the study, intervention (name of probiotics, dosage and frequency of administration), maintenance/induction of remission, results of the trial concerning our primary objective, effect size, type of evaluation of the intervention (clinical/endoscopic/histological assessment) and time until recurrence. The data were documented in forms by two blindly to each other working reviewers (EK, MM); in case of disagreement, a third reviewer (ML) mediated for its settlement. The Newcastle-Ottawa scale was applied to estimate the risk of bias for the selected non-randomized studies whereas for the randomized trials, the Risk of Bias 2 (RoB2) tool of Cochrane was used. The primary outcome was the achievement of maintenance of remission, expressed either as risk of recurrence and alterations in CDAI or other activity scores, such as Rutgeert's score. In cohort studies, 6 months were considered sufficient median time for the follow-up of the participants.

4. Results

Selection and Description of Studies

The search strategy in PubMed and Cochrane retrieved 2015 articles. Of these, 7 articles were removed as duplicates and 2008 articles were screened in title. 1975 articles were excluded as irrelevant, 32 were assessed for eligibility in full text with 18 of them being excluded after careful consideration, as presented in the Supplemental Table 1. Overall, 11 studies were included in the final analysis, with 952 patients with CD participating in them. The aforementioned stages are elucidated in Figure 2. The results of this Systematic Review are summarized in the Supplemental Table 2.

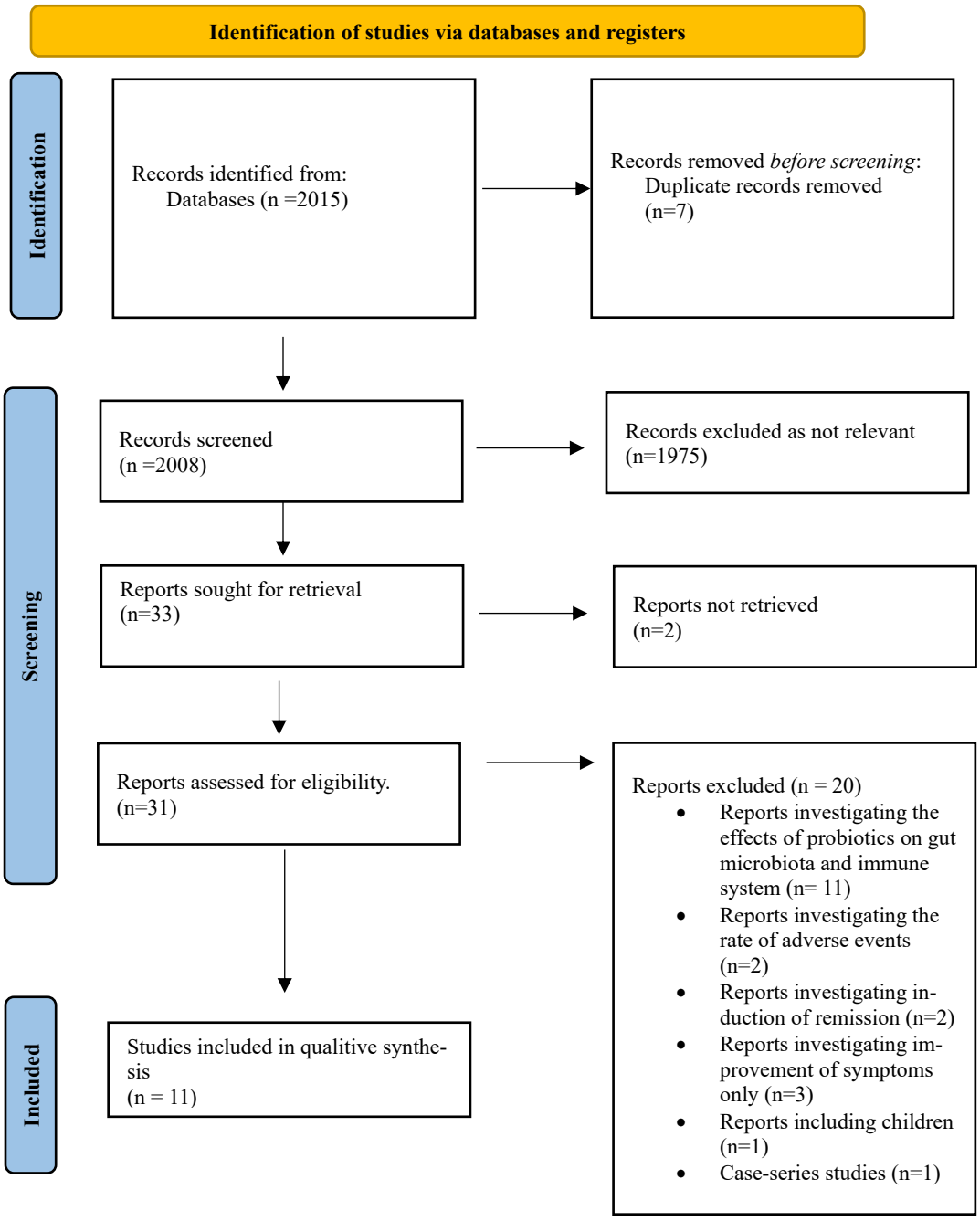

***Figure 2**: PRISMA Flow-diagram of this Systematic Review*

9 studies were randomized clinical trials, with 6 of them being double-blind and 3 open-label. The remaining 2 studies were cohort studies. All trials investigated the use of probiotics, either alone or as synbiotics, for the preservation of remission in CD. Five studies focused on patients who had recently undergone surgery for their disease. Totally, 822 patients with CD were included in the present review, with 363 patients having undergone surgical intervention for their disease and 459 being surgically naïve. 200 patients with UC were included in 4 trials. Of note, in 3 trials the proportion of patients with UC was greater than those with CD - 88 vs 28 patients in the study of Tan et al, 31 vs 9 patients in the trial of Fan et al and 81 vs 62 patients in the study of Bjarnason et al.

Utilized Activity Scores

Concerning the activity score, CDAI score was applied to 6 trials, with the baseline being less than 150 in 4 studies, between 150 and 300 in 1 whereas the baseline score was not mentioned in 1 trial. Rutgeert's score was employed in 3 studies and Harvey-Bradshaw index in 2. Notably, the study conducted by Tan F. et al, which included patients with UC and CD, utilized the Clinical Activity Index (CAI) and the Endoscopic Activity Index (EAI) without providing further information. Unfortunately, in 3 studies the baseline CDAI score was not mentioned. All patients who underwent surgical management of their CD in the eligible trials had an *i0/c0* Rutgeert's score at baseline. Finally, concerning the Harvey-Bradshaw index, the study held by Bjarnason I. Et. Al included patients with a score less than 5.

Investigated Probiotics/Synbiotics

Among the probiotics, *Lactobacillus* species were included in 8 studies, in 4 as a monotherapy. Following Lactobacillus, *Bifidobacterium* was investigated in 6 studies of which 4 contained the combination of *Bifidobacterium* species with Lactobacillus (3), *Enterococcus species* (2) and *Streptococcus* (1). *Saccharomyces boulardii* was explored in 2 of 14 trials. The effect of synbiotics was explored in 2 clinical trials: Synbiotic 2000, consisting of 4 lactic acid bacteria and 4 fermentable fibers, and the combination of Bifidobacterium longum (probiotic) and Synergy 1 (prebiotic).

Follow-up Periods

The follow-up time of the included studies was identical to the administration of the investigated pro-/symbiotic, ranging from 1 month to 2 years. The shortest follow-up time was 1 month as it was observed in the studies of Bjarnason et. Al and Fan et al. On the other hand, the longest follow-up time was 24 months as noted in the study of Chermesh I., while the trials of Bourreille A. et al, Fedorak RN et al and Prantera C. et al required 1 year of follow-up.

Results on Maintenance of Remission from Cohort Studies

The study conducted by Tan F. et al reviewed retrospectively the 2-month use of Bifida triple, a probiotic containing Bifidobacterium longum, Lactobacillus acidophilus and Enterococcus faecalis, in a cohort of patients with IBD. The proportion of CD was limited as only 28 patients suffered from CD. All participants received Bifida triple 3 times a day for 2 months together with mesalazine. The effectiveness of this therapeutic scheme was assessed by symptomatic relief and endoscopic healing, thus employing the Clinical Activity Index (CAI) and Endoscopic Activity Index (EAI), and subsequently calculating the total effective rate [(number of cases with complete symptoms' alleviation and endoscopic healing + cases of partial symptoms' alleviation and endoscopic improvement)/the total number of patients]. The treatment group,

comprising of patients with CD and UC, demonstrated a total effective rate of 88.52% compared to 70.91% of the control group ($p<0.05$) at the end of the 2-month follow-up period.

The retrospective cohort study held by Oh GM et al examined the concomitant use of Saccharomyces boulardii with aminosalicylates (93.4%), thiopurines (82.2%) and biologic agents (20.4%) in 152 patients with CD in remission (CDAI <150). After comparing the total CDAI scores at the beginning of treatment and after 6 months, a statistically significant difference ($p<0.01$) was detected as the total CDAI score decreased from 40.65 (± 53.65) to 32.06 (± 50.29).

Results on Maintenance of Remission from Randomized Trials

Guslandi et al first explored the efficacy of Saccharomyces boulardii combined with mesalazine versus mesalazine alone in their open-label trial. After 6 months of treatment, 1 patient of 16 in the intervention arm experienced clinical relapse whereas 6 patients of 16 in the mesalazine group had CDAI score over 100 ($p=0.04$).

Saccharomyces boulardii was also investigated in the study of Bourreille et al comparing with placebo its efficacy at reducing the risk of relapse after 52 weeks of treatment. In total, 38 of 80 patients (47.5%) in the S boulardii group had relapse at week 52 comparing to 42 of 80 patients (53.2%) in the placebo group ($p=0.5$), with the results being similar after stratification according to the medication used for induction of remission. After adjustment on the CDAI at randomization and on the stratification factor, the mean CDAI alterations from the beginning at the end point were 79.7 and 69.0 points in the treatment and control groups, respectively, with the effect of the stratification factor not being statistically significant ($p=0.99$). Importantly, after performing a post-hoc analysis on the association of baseline characteristics with the relapse rate, it was indicated that the presence of extraintestinal manifestations was the only statistically significant parameter over corticosteroids as an induction therapy and the location of disease. Finally, the effect of smoking on disease recurrence was evaluated in each arm. In the S. boulardii arm, smokers had higher a recurrence rate of 54.5% compared to 34.5% of nonsmokers. Nonetheless, in the placebo group, smokers had lower recurrence rates (48.5%) relative to nonsmokers (72%).

The randomized, double-blind, placebo-controlled trial reported by Bjarnason et al investigated the use of Symprove, a multi-strain probiotic containing Lactobacillus and Enterococcus, primarily in the improvement in the quality of life of patients with IBD and, secondarily, in the difference in clinical activity scores between the 2 arms before and after the 4-week study period. Despite including patients with UC, the investigators analyzed separately for UC and CD the changes in the Harvey-Bradshaw Index without finding a difference in the total HBI score at the end of the study [Mean difference: 0.2 (− 1.0, 1.4), $p= 0.66$]. No differences were found in the quality of life between the 2 arms, irrespective of their disease.

The probiotic Bifico, comprising of Bifidobacterium, Lactobacillus acidophilus, and Enterococcus faecalis, was studied as a combination with extended action form of Mesalazine (Pentasa) in the open-label trial of Fan et al. In the study, which included patients with UC, it was observed that there is a statistically significant (p=0.0233) decrease in the CDAI scores between the Bifico-Pentasa and Pentasa alone groups (3.86±2.16 in treatment arm versus 5.29±2.48 in control). The recurrence rate was statistically lower in the treatment arm as only 1 of 21 participants (4.76%) in it experienced recurrence versus 6 of 19 (31.58%) in the control group ($p=0.0395$).

In the surgical setting, the trial of Fedorak et al investigated the role of the probiotic VSL#3 in the endoscopic recurrence of CD patients who had ileocolonic resection with a small-intestine-to-colon anastomosis. It is important to state that the baseline CDAI scores were over 150 (for VSL#3 169.7 ± 83.1 and for placebo: 164.8 ±81.4, *p=0.74*). This commixture of 8 different bacteria among the Lactobacillus, Bifidobacterium and Streptococcus species was explored via a double-blind phase followed by an open-label phase. Of the 120 originally enrolled patients, 57 patients completed the 365-day study period. At the end of the double-blind phase (3 months), the rates of endoscopic recurrence were analogous among the 2 groups as in the VSL#3 32 of 43 patients (74.4%) experienced some kind of endoscopic recurrence whereas in the placebo group, 39 of 51 (76.5%, *p=0.82*). The number of patients remaining in lower grades of endoscopic recurrence was similar, as well, with 43 of 58 participants (74.1%) receiving VSL#3 compared to 39 of 62 (62.9%) in the placebo group. During the open-label phase, 10% (3/30) of the previously treated VSL#3 arm experienced severe endoscopic recurrence as to 26.7% (8/30) of the previous placebo arm (*p=0.09*). Finally, from the cohort of patients who completed all sections of this study, 89.6% (26/29) of the VSL#3-initially-treated people experienced a lower-grade endoscopic recurrence (grade 0, 1, 2) whereas in the placebo-initially-treated arm the percentage was 71.4% (20/28). In summary, although not statistically significant, the results indicated a trend favoring the early initiation of VSL#3 in patients with CD and recent ileocolonic resection aiming at averting severe endoscopic recurrence.

Contradictory results were shown by Chermesh et al in the setting of postoperative recurrence of CD. Synbiotic 2000, comprising of 4 lactic acid bacteria of the Lactobacillus genus and 4 fermentable fibers, was administered to 30 patients with recent surgical interventions for their disease. The participants were evaluated endoscopically 3 months after their surgery and at the end of the trial (24 months) or in the scenario of recurrence. In the first endoscopic assessment, the Rutgreets' scores were not statistically significant between the intervention and placebo group (0.6±0.8 vs 0.8±1). 5 of 20 patients in the Synbiotic arm experienced disease recurrence relating to 3 of 10 patients in the placebo arm. Of note, only 9 patients fulfilled the 24-month trial, 7 of them being from the Synbiotic arm and 2 from the placebo one.

The effectuality of probiotics, and especially Lactobacillus GG (LGG), in the postoperative setting was appraised in the trial of Prantera et al. Patients with recent curative resection for their CD were randomized to receive either LGG or placebo for 52 weeks aiming at maintaining endoscopic remission at 12 months or decreasing the severity of recurrent lesions. The assessment was performed through the Rutgeerts endoscopic score and the calculation of CDAI in each visit. The outcomes revealed that 15 of 18 (83.3%) patients in the LGG group remained in clinical remission, with a CDAI score less than 150, 1 year after the initiation of the study compared to 17 of 19 (89.4%) in the placebo group. In the endoscopy of these patients, 60% (9/15) had recurrent endoscopic lesions while the percentage in the placebo group was only 33.5% (6/17, *p=0.297*). The remaining patients had severe endoscopic recurrence in both groups; 6/15 in the LGG arm (40%) and 3/17 in the placebo arm (17.6%), *p=0.313*. All results did not meet the desired statistical significance.

Similar results were demonstrated by the double-blind, placebo-controlled study of Marteau et al which investigated the value of Lactobacillus johnsonii LA1 in the postsurgical recurrence of CD. The investigators administered LA1 for 6 months to patients with recent surgery for CD, evaluating the disease activity with CDAI at each study visit and with endoscopy, employing the Rutgeert's score, at the end of the study or earlier, in the scenario of recurrence. Endoscopic recurrence of any grade was seen in 49% (21/43) in

the intervention group versus 64% (30/47) in the placebo arm [OR 1.85 (95% CI 0.80–4.30), *p=0.15*] while severe recurrence was met in 21% (9/43) in the intervention arm compared to 26% (12/47) in the placebo arm [OR 1.30 (95% CI 0.48–3.47), *p=0.61*].

Another study which examined the efficacy of Lactobacillus johnsonii (LA1) after ileo-caecal resection was executed by Van Gossum et al. This double-blind, placebo-controlled trial was designed to primarily evaluate the endoscopic recurrence of CD 12 weeks postoperatively and secondarily the clinical relapse rate with the use of CDAI. Both in ITT and PP analysis and after 3 months of treatment, no significant difference in the mean endoscopic score was found between the two arms (LA1 versus placebo [n = 28 versus n = 27]: 1.50 ± 1.32 versus 1.22 ± 1.37, treatment effect*: p= 0.48*). Calculated at each visit, CDAI was not significantly modified (treatment effect: *p= 0.67*, visit effect *p= 0.004*; treatment and visit interaction*: p= 0.10,* mixed model) as it was not the mean histological score (LA1 versus placebo: 4.58 ± 2.82 versus 3.73 ± 2.19, treatment effect *p= 0.83*, mixed model after log-transformation).

Inflammatory Markers

As far as the inflammatory markers are concerned, 7 of the included studies investigated the effect of probiotics in certain inflammatory markers, mainly consisting of CRP in 6 of 7 studies, ESR in 3 studies and fecal calprotectin in 2 studies. The study of Tan et al included TNF-a and IL-6 as inflammatory indexes while IL-6 together with IL-4 were examined in the trial of Fan et al. The study of Oh et al encompassed ferritin, too, whereas the study of Fan et al incorporated fecal lactoferrin in their investigations.

Positive results were met in 3 studies. The study of Tan et al reported statistically significant reductions in the values of TNF-a, IL-6 and CRP in both groups with greater reductions being noted in the intervention group. Similar results were demonstrated in the trial of Fan et al in which the levels of hs-CRP, fecal lactoferrin and IL-6 were significantly lower in the treatment group whereas IL-4 was significantly greater in the probiotic arm. The trial of Chermesh et al, although it failed to demonstrate the effect of Synbiotic 2000 in the postoperative maintenance of remission, indicated that the levels of ESR were lessened in both groups after 3 months of follow-up but this reduction did not maintain its statistical significance 24 months post-surgery.

On the other hand, in the remaining 4 studies, the alterations of inflammatory markers did not reach the level of statistical significance. The trials of Bjarnason et al, exploring the kinetics of CRP, ESR, fecal calprotectin, white blood cells count, Bourreille et al and Van Gossum et al, both of them investigating CRP and ESR between groups, did not manage to extract significant results while the baseline values in the intervention and placebo groups were similar. Notably, the study of Oh et al indicated an increase in ferritin, CRP and fecal calprotectin levels, despite the promiscuous results of probiotics in CDAI. All the aforementioned results are summarized in Table 3.

Performed Surgical Interventions

Regarding the 5 trials held in the postoperative setting, 4 trials reported the type of resection while the report of Chermesh et al the type of surgical intervention was not mentioned. The trials of Marteau et al and Prantera et al incorporated 3 different types of surgery (ileal, ileocolonic and colonic) with the most frequent being the ileocolonic and ileal, respectively. Fedorak et al included patients with ileocolonic resection only while Van Gossum et al referred only to patients with recent ileoceacal resection.

The initial behavior of the disease leading to surgery was variable among the studies, with the exception of the Fedorak et al trial in which it is not stated. Fibrostenotic disease with obstructive events were the primary indication for surgical intervention in the trials of Van Gossum et al and Prantera et al. Inflammatory behavior was predominant in the trial of Chermesh et al with the minority of patients in both arms exhibiting a non-inflammatory course of disease. A more penetrating nature was seen in the study of Marteau et al with 50% of the placebo and 42% of the probiotic group demonstrating fistulas, abscesses or acute free perforation. The results are summarized in Table 4.

Quality of Life

The effect of probiotics' administration in the quality of life of CD patients was examined in 2 of the included trials. The investigators employed the IBD Quality of Life Questionnaire (IBDQ) which includes 32 questions regarding systemic symptoms (S), bowel symptoms (B), emotional (E) and social (SF) function. The results from the study of Bjarnason et al did not show any alterations before and after treatment and between the 2 arms. In the study of Fedorak et al, the investigators reported that the IBDQ scores did not distinct between the 2 treatment groups at 3 and 12 months of follow-up. The results are stated in Table 3.

Gut microbiome alterations

The study of Fan et al was the sole trial to investigate the effect of probiotics, specifically Bifico, on microflora structure. The writers analyzed stool samples from the participants, prior and after the interventions. Although statistical significance was not met, findings suggested that the quantity of 4 phyla, the ones of Enterobacteria, Enterococci, Bacteroides and Saccharomyces, was reduced while the number of Bifidobacteria and Lactobacilli was raised.

Risk of Bias

The evaluation of risk of bias is presented in the Supplemental Tables 6 and 7. Concerning the 2 cohort studies, both studies had low risk of selection bias as per the Newcastle-Ottawa Quality Assessment Scale. However, as far as the baseline measurements of the outcome of interest are concerned, the baseline scores in the study of Tan et al were presented graphically but not analytically throughout the text and could not be accurately extracted. Despite attempts to contact the authors, the data was not available for our analysis.

Respecting randomized controlled trials, all trials had low risk of bias in the fields of the randomization process and measurement of the outcome. However, in 3 studies there were some concerns on the selection of the reported results and any deviations from intended treatments, as per the Risk of Bias Assessment Tool (RoB2). Finally, in the study of Chermesh et al some concerns were raised on the risk of bias in the field of missing outcome data predominantly because of the lack of reporting detailed strategies for handling missing data. The reasons for dropout are provided, which helps in understanding potential biases, but the lack of detailed handling of missing data in the analysis adds to the concern.

5. Discussion

Probiotics have immunomodulatory effects on the gastrointestinal system, creating, therefore, an assumption on their ability to sustain remission in IBD. To date, guidelines do not suggest probiotics as an additional factor in inducing or maintaining remission of the disease[56]. Taking into account their widespread availability, low-cost and relatively restricted side effects' profile, their use in the maintenance of quiescent Crohn's disease poses a valid option. Unfortunately, throughout literature, it has not universally been proven that probiotics may affect the remission of CD.

The mainstay of treatment for the maintenance of remission is the use of biologic agents, immunomodulators and corticosteroids. It is noteworthy that, according to the consensus published by the European Crohn's and Colitis Organisation (ECCO), 5-aminosalicylates are not recommended as a sole option for the maintenance of remission in CD[57]. To prevent postsurgical recurrence, the risk of recurrence must be calculated and stratified according to the age, the duration of the disease, the number of prior surgeries and the type of disease's behavior (perforating, fibrostenotic, non-stricturing and non-perforating). It has been found that perforating course of CD presents with a more aggressive course of relapse, indicating higher chances of relapse[58,59]. The initial disease behavior predicts the type of reoccurrence[60]. Accordingly, the consideration of the appropriate medication should be held bearing in mind several assorted factors. Anti-TNF therapy may be selected in either low or high risk, while antibiotics remain an alternative in intermediate risk.

In our review we investigated the augmentation of probiotics in the maintenance of remission in Crohn's disease. Only 4 of the 12 involved studies indicated a trend favoring the use of probiotics in the maintenance of CD. Specifically, the studies of Tan et al, Guslandi et al, Oh et al and Fan et al reported a statistically significant outcome, utilizing the combination of Bifidobacterium, Lactobacillus and Enterococcus in 2 studies and Saccharomyces boulardii in the other 2. In the remaining studies, no effect of statistical importance was found. It has been demonstrated in included randomized studies both in the non- and post-operative setting, that the administration of Lactobacillus strains alone is not an optimal alternative. Notably, all studies held in the post-operative setting, in which there was not concomitant administration of other medication, did not reveal any benefit. The evidence suggests that, while some probiotics strains may have potential benefits in this clinical setting, the overall effectiveness varies among studies.

The variability of our findings highlights the complexity of the right choice of probiotic and clinical setting for CD, suggesting that the application of probiotics in the treatment of CD may need to be personalized. This is supported by the analysis of Xia, Y et al which indicated that microbial structure and function is altered not only between IBD patients and healthy individuals but also among different geographic regions[61]. The precise mechanisms through which probiotics might exert beneficial effects in CD remain an area of active investigation. Some of the proposed mechanisms of their action include the decrease of mucosal inflammation through the modulation of T-cell activity, the recovery of mucosal barrier integrity, inhibiting, therefore, the stimulation of the epithelial cells from antigens and the readjustment of intestinal microflora, with reduction in the number of pathogenetic species[62,63]. Moreover, the interaction between probiotics and the host microbiome is highly individualized, suggesting that genetic, environmental, and lifestyle factors could significantly influence treatment outcomes.

Apart from this, the concomitant use of traditional therapy together with personalized probiotic scheme could be a new therapeutic strategy. Specific strains, such as Bifico (Bifidobacterium longum, Lactobacillus acidophilus and Enterococcus faecalis) and S. boulardii, may prove beneficiary indicating potential targets for future interventions always together with the administration of other agents, such as mesalazine and biologic agents. Together with aminosalicylates and the other therapies currently indicated for the maintenance of remission, a synergistic effect is theoretically achieved and therefore a benefit may be derived for the patient. Despite these, in the majority of the included trials, no effect over the maintenance of remission was demonstrated with the use of probiotics either as monotherapy or as concomitant medicine.

Similar to our findings, the introduction of probiotics in Crohn's disease has not been proved beneficial neither in the induction nor in the maintenance of remission in the meta-analysis conducted by Fujiya et al [64]. Accordingly, Chen et al displayed unfavorable outcomes for probiotics as an adjunctive factor for the maintenance of CD, although positive results were shown for the induction of remission in UC[65] . Consistently, 2 meta-analyses conducted by Zhang et al and Vakadaris et al indicated poor effectiveness of probiotics in CD, with, however, overall positive results in inducing and maintaining remission in IBD[54,66]. Contrastingly, encouraging results about the use of probiotics in Crohn's disease were demonstrated by Ganji-Arjenaki M. and Rafieian-Kopaei M. in their systematic review and meta-analysis indicating that probiotics may be efficient in CD, especially after surgery[67].

In our review, only 1 trial elaborated on the use of synbiotics. The introduction of synbiotics in the treatment of CD represents another promising area of research. Prebiotics may enhance the effects of probiotics by creating a more favorable gut environment. Future studies should explore the potential synergistic effects of synbiotics in the maintenance of Crohn's disease remission.

The study by Fan et al. uniquely explored the impact of Bifico, a probiotic combined with Pentasa, on gut microflora. The authors observed changes in the abundance of several key microbial phyla. Specifically, there was a reduction in the quantities of Enterobacteria, Enterococci, Bacteroides, and Saccharomyces, and an increase in Bifidobacteria and Lactobacilli levels. Although these changes did not reach statistical significance, they suggest a potential trend towards a more balanced gut microbiota composition, typically associated with improved gut health. The increase in beneficial bacteria such as Bifidobacteria and Lactobacilli is particularly noteworthy. These bacteria are known to play crucial roles in maintaining intestinal health by inhibiting pathogenic microbes, enhancing the intestinal barrier function, and modulating immune responses. The observed reduction in potentially harmful bacteria like Enterobacteria and Enterococci further supports the beneficial impact of Bifico on gut health. While this study suggests that Bifico may positively influence gut microbiota by increasing beneficial bacteria and reducing potentially harmful ones, the results are preliminary.

One explanation for these rather disheartening results of our review could be the diversity of the GI microbial environment among patients, which has been affected by the intestinal inflammation as well as the surgical removal of the inflamed areas and may be susceptible to different probiotics. It is known that the postoperative gut microbiota presents distinct differences from healthy subjects. According to the systematic review of Zhuang X et al, patients with post-operative recurrence exhibited restricted diversity in the ileal mucosal microbiome. What is more, the review found that certain phyla, such as the Proteobacteria ones, were highly expressed whereas others, such as Bacteroidetes and Firmicutes phyla, were not abundant[68].

As probiotics tend to amend the imbalance within the intestinal microflora by improving the quantity and quality of protective bacteria, there is a rationale in their use in IBD. However, considering their inability to induce positive results despite the anti-inflammatory actions, the reasons behind it should be also sought in the intestinal wall, their pharmacodynamics and pharmacokinetics[69,70]. Herein, we need to further elucidate the mechanisms of the phenomenon of *dysbiosis* and the input of probiotics in the amelioration of inflammation.

The follow-up periods of the included studies varied significantly, ranging from 1 month to 2 years. Shorter follow-up periods, as seen in the studies by Bjarnason et al. and Fan et al, can provide initial insights into the immediate impact of probiotics. However, safe conclusions on the long-term effects, risks and benefits cannot be drawn. On the other hand, longer follow-up periods, as noted in the study by Chermesh et al, may prove more useful for assessing the sustainability of remission and long-term safety of probiotics as they provide a more comprehensive understanding of the potential enduring effects and can better capture any delayed adverse events or benefits that might not be apparent in shorter studies. Any follow-up period which falls between these offers a balance, allowing for the observation of medium-term outcomes and providing valuable insights into the efficacy and safety of probiotics over a longer period than the short-term studies, but not as extensively as the 24-month study. The range of follow-up periods highlights the variability in study designs and the potential implications for interpreting the efficacy of probiotics in maintaining remission in Crohn's disease. Studies with shorter follow-up may underestimate or miss long-term benefits or adverse effects, while those with longer follow-up provide a more thorough assessment but are fewer in number. Longer follow-up periods are essential for understanding the chronic nature of Crohn's disease together with the role of probiotics in sustained remission.

The investigation into the effects of probiotics on inflammatory markers in Crohn's disease (CD) patients yielded mixed results across the seven included studies. The markers studied predominantly included C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and fecal calprotectin, with some studies also examining cytokines such as TNF-α, IL-6, IL-4, and other markers like ferritin and fecal lactoferrin.

Three studies reported positive outcomes. Tan et al. observed statistically significant reductions in TNF-α, IL-6, and CRP levels in both the intervention and control groups, with greater reductions noted in the probiotic group. Similarly, Fan et al. reported significant decreases in hs-CRP, fecal lactoferrin, and IL-6 levels, alongside an increase in IL-4 in the probiotic group. Chermesh et al., although not showing a significant effect of Synbiotic 2000 on postoperative remission maintenance, found a temporary reduction in ESR levels at 3 months, which was not sustained at 24 months. In contrast, the remaining four studies did not find significant changes in inflammatory markers. Bjarnason et al. investigated CRP, ESR, fecal calprotectin, and white blood cell counts, while Bourreille et al. and Van Gossum et al. focused on CRP and ESR. All three studies reported no significant differences between the probiotic and control groups. Interestingly, Oh et al. noted an increase in ferritin, CRP, and fecal calprotectin levels, despite improvements in the Crohn's Disease Activity Index (CDAI), highlighting a possible discrepancy between clinical symptoms and inflammatory marker levels.

These disparate findings can be attributed to several factors. Once again, variations in the probiotic strains, dosages, and duration of treatment might play a crucial role in influencing outcomes. Differences in study populations, including disease severity, baseline inflammatory marker levels, and concomitant therapies,

could also impact results. Furthermore, the timing of marker assessment post-treatment might affect the observed effects, as inflammatory responses can fluctuate over time.

The limited and inconsistent evidence underscores the need for more standardized and rigorous research protocols. Future studies should aim for uniformity in their protocols so as to evaluate the impact of probiotics on inflammatory markers. In summary, while some studies suggest that probiotics might beneficially impact certain inflammatory markers in CD patients, the overall evidence remains inconclusive. A more consistent approach in future research could help clarify the potential role of probiotics in managing inflammation in Crohn's disease, ultimately guiding more effective clinical applications.

In some trials occurring at the postoperative setting, probiotics were used as monotherapy. As the factors contributing to the development of IBD are complex, the combination of probiotics with standard treatment schemes may prove of benefit directing at suppressing the increased immune response while improving the mucosal barrier function and the composition of the intestinal microflora. Finally, throughout the involved studies, it was recognized that there was not a standardized formulation, dosage, scheme of administration, duration of treatment and follow-up period, even in studies employing the same probiotic, which may explain the discrepancy among the results.

The variability in the type of surgical interventions and the initial disease behavior leading to surgery across the reviewed trials underscores the complexity of Crohn's disease management and its impact on the outcomes of probiotic use in maintaining remission. The trials of Marteau et al. and Prantera et al. included a broader range of surgical types (ileal, ileocolonic, and colonic), with ileocolonic and ileal resections being the most frequent. In contrast, Fedorak et al. and Van Gossum et al. focused on more specific surgical populations, limiting their subjects to ileocolonic and ileocecal resections, respectively. This heterogeneity in surgical types might influence the applicability of the findings, as the postoperative recurrence and response to probiotics could vary depending on the location and extent of the resection.

The initial behavior of the disease leading to surgery also varied significantly among the studies. While fibrostenotic disease was the primary indication in the trials of Van Gossum et al. and Prantera et al., Chermesh et al. predominantly included patients with an inflammatory disease course. Additionally, Marteau et al. reported a higher incidence of penetrating disease in their cohort. These differences are crucial, as they may impact the underlying pathophysiology and microbiota composition, potentially affecting the efficacy of probiotics in maintaining remission. In conclusion, while the reviewed trials provide valuable insights into the potential role of probiotics in maintaining remission in Crohn's disease, the heterogeneity in surgical types and disease behaviors must be carefully considered. A more uniform approach in future research could enhance our understanding and potentially lead to more tailored probiotic interventions for CD patients who have undergone surgical treatment.

Apart from randomized trials which are crucial to the establishment of the role of probiotics in CD, real-world evidence from observational studies and patient registries can provide valuable insights into the long-term use of probiotics in clinical practice. These studies can help identify factors that influence adherence, effectiveness, and patient satisfaction. Long-term follow-up is crucial to understanding the sustainability of probiotic benefits and any potential delayed effects.

The World Health Organisation (WHO) defines quality of life (QoL) as “an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their

goals, expectations, standards and concerns"[71]. Patients suffering from IBD frequently experience a variety of symptoms either related to their disease or to their treatment, with these negatively influencing their well-being, personal and social life. The systematic review of Mitropoulou et al displayed that people with IBD have signs of anxiety and depression which lead to lower quality of life in terms of intestinal and systematic symptoms, keeping in mind that poorer psychological status is correlated to the disease's symptoms and reduced treatment response[72,73].

The impact of probiotic administration on the quality of life (QoL) in Crohn's disease (CD) patients was specifically assessed in two of the included trials, utilizing the Inflammatory Bowel Disease Quality of Life Questionnaire (IBDQ)[74]. This questionnaire, encompassing systemic symptoms (S), bowel symptoms (B), emotional (E), and social function (SF), provides a comprehensive evaluation of patient well-being.

The trial conducted by Bjarnason et al. found no significant differences in IBDQ scores before and after treatment, nor between the probiotic and control groups. Similarly, Fedorak et al. reported no discernible differences in IBDQ scores between the treatment groups at both 3 and 12 months of follow-up. These consistent findings across studies suggest that probiotics, at least as administered in these trials, do not significantly enhance the quality of life for CD patients in remission. Several factors could account for these results. The strain and dosage of probiotics, as well as the duration of administration, may not have been sufficient to elicit a measurable improvement in QoL. Additionally, the baseline quality of life of participants and the presence of other confounding variables, such as concurrent medications and disease severity, could have influenced the outcomes. It is also possible that the QoL improvements due to probiotics might be subtle or occur over a longer period than the follow-up durations of these studies.

These findings highlight the need for further research to determine the optimal probiotic strains, dosages, and treatment durations that might yield more pronounced benefits. Moreover, future studies should consider stratifying participants based on baseline QoL and other relevant factors to better identify subgroups that might benefit more from probiotic therapy. Finally, while the current evidence does not support a significant impact of probiotics on the quality of life in CD patients, these findings should be interpreted with caution.

Finally, the study by Bourreille et al. provides intriguing insights into the role of smoking status on the effectiveness of S. boulardii in preventing relapse in Crohn's disease (CD) patients. The findings highlight a complex relationship between smoking, probiotic treatment, and relapse rates, which warrants careful consideration. In the treatment arm, nonsmokers had lower recurrence rates, suggesting that S. boulardii might be more effective in nonsmokers. On the contrary, in the placebo arm, nonsmokers on placebo experienced a higher relapse rate compared to smokers and former smokers. This surprising result suggests that factors beyond probiotic treatment and smoking status alone might be influencing relapse rates. Smoking has been associated with higher risk of complications, surgery and more frequent episodes of relapse. Furthermore, patients-smokers have higher chance of requiring corticosteroids, immunosuppressive and surgical therapy than non-smokers. Finally, the permanent cessation of smoking advances the course of CD[75].

Limitations

This systematic review has some limitations. Firstly, a limitation of our review is the inability to extract baseline data from certain studies, such as the study of Tan et al. This may impact our ability to fully assess

the comparability of groups at baseline and introduces uncertainty in the risk of bias assessment. The lack of detailed baseline data highlights the need for more complete reporting in primary studies. Furthermore, another limitation in assessing therapeutic efficacy in some studies is the requirement for both symptom alleviation and endoscopic improvement to classify a treatment as effective. This may lead to patients who experience symptomatic relief but no endoscopic improvement being categorized as 'Ineffective', thereby affecting the reported efficacy rates. Additionally, there were studies with small cohorts of CD patients. Lastly and as stated before, the disparities in treatment designs among the included studies affect the consistency of the indicators of efficacy.

Some limitations may be found in the studies in the postoperative period. Specifically, the absence of detailed surgical intervention reporting in the study by Chermesh et al. limits the ability to fully compare its findings with those of other trials. Moreover, the variation in disease behavior and surgical types across studies complicates the generalizability of the results. Future studies should aim for more standardized reporting and stratification based on disease phenotype and surgical intervention to better understand the role of probiotics in different subpopulations of Crohn's disease patients.

Conclusion

In this systematic review, the contribution of probiotics in the maintenance of remission in Crohn’s disease, either as a monotherapy or as adjuvant medication, could not be established. Future research should focus on large-scale, randomized controlled trials with standardized probiotic formulations and longer follow-up periods to better assess long-term efficacy. Additionally, studies exploring the microbiome profiles of responders versus non-responders could provide valuable insights into personalized probiotic therapy for Crohn's disease. Personalized probiotic therapies, guided by further research, may offer a viable adjunctive treatment for Crohn's disease remission maintenance.

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Supplemental Table 1: Excluded studies.

Author	Title	Journal, Issue, Page
Reports investigating the improvement of symptoms		
Tomita T.	Effect of Bifidobacterium bifidum G9-1 on the Intestinal Environment and Diarrhea-Predominant Irritable Bowel Syndrome (IBS-D)-like Symptoms in Patients with Quiescent Crohn's Disease: A Prospective Pilot Study	Journal of Clinical Medicine vol. 12,10 3368. 9 May. 2023
Hedin CR	Probiotic and prebiotic use in patients with inflammatory bowel disease: a case-control study	Inflammatory bowel diseases vol. 16,12 (2010): 2099-108
Bonavina L.	Abincol® (Lactobacillus plantarum LP01, Lactobacillus lactis subspecies cremoris LLC02, Lactobacillus delbrueckii LDD01), an oral nutraceutical, pragmatic use in patients with chronic intestinal disorders	Acta bio-medica: Atenei Parmensis vol. 90,7-S 8-12. 10 Jul. 2019
Reports investigating the effects of probiotics on gut microbiota and immune system		
Shadnoush M.	Probiotic yogurt Affects Pro- and Anti-inflammatory Factors in Patients with Inflammatory Bowel Disease	Iranian journal of pharmaceutical research, JPR vol. 12,4 (2013): 929-36
Paroni M.	An intestinal Th17 subset is associated with inflammation in Crohn's Disease and activated by adherent-invasive Escherichia coli (AIEC)	Journal of Crohn's & colitis vol. 17,12 (2023): 1988-2001
Garcia Vilela E.	Influence of Saccharomyces boulardii on the intestinal permeability of patients with Crohn's disease in remission	Scandinavian Journal of Gastroenterology vol. 43,7, 2008
Ballini A	Probiotics Efficacy on Oxidative Stress Values in Inflammatory Bowel Disease: A Randomized Double-Blinded Placebo-Controlled Pilot Study	Endocrine, metabolic & immune disorders drug targets vol. 19,3 (2019): 373-381
Yılmaz İ	Effect of administering kefir on the changes in fecal microbiota and symptoms of inflammatory bowel disease: A randomized controlled trial	The Turkish journal of Gastroenterology : the official journal of Turkish Society of Gastroenterology vol. 30,3 (2019): 242-253
Lorea Baroja M	Anti-inflammatory effects of probiotic yogurt in inflammatory bowel disease patients	Clinical and experimental immunology vol. 149,3 (2007): 470-9. doi:10.1111/j.1365-2249.2007.03434
Ahmed J	Impact of probiotics on colonic microflora in patients with colitis: a prospective double blind randomised crossover study	International journal of surgery (London, England) vol. 11,10 (2013): 1131-6
Shadnoush M.	Effects of Probiotics on Gut Microbiota in Patients with Inflammatory Bowel Disease: A Double-blind, Placebo-controlled Clinical Trial	The Korean journal of gastroenterology = Taehan Sohwagi Hakhoe chi vol. 65,4 (2015): 215-21
Su H.	Effects of glucocorticoids combined with probiotics in treating Crohn's disease on inflammatory factors and intestinal microflora	Experimental and therapeutic medicine vol. 16,4 (2018): 2999-3003

Bodini G.	Reduction of Fecal Calprotectin Levels Induced by a Short Course of Escherichia Coli Nissle is Associated with a Lower Likelihood of Disease Flares in Patients with Ulcerative Colitis in Clinical Remission	Journal of gastrointestinal and liver diseases: JGLD vol. 32,4 438-443. 22 Dec. 2023
Bamola VD	Role of a probiotic strain in the modulation of gut microbiota and cytokines in inflammatory bowel disease	Anaerobe vol. 78 (2022): 102652
Reports investigating induction of remission		
Steed H.	Clinical trial: the microbiological and immunological effects of synbiotic consumption - a randomized double-blind placebo-controlled study in active Crohn's disease	Alimentary pharmacology & therapeutics vol. 32,7 (2010): 872-83
Schultz M.	Lactobacillus GG in inducing and maintaining remission of Crohn's disease	BMC Gastroenterol. 2004; 4:5. Published 2004 Mar 15
Reports investigating adverse events		
Braat H.	A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease	Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association vol. 4,6 (2006): 754-9
Dore MP	Effect of Probiotic Use on Adverse Events in Adult Patients with Inflammatory Bowel Disease: a Retrospective Cohort Study	Probiotics and antimicrobial proteins vol. 12,1 (2020): 152-159
Reports including pediatric patients		
Nousiainen P.	Complementary and alternative medicine use in adolescents with inflammatory bowel disease and juvenile idiopathic arthritis	MC complementary and alternative medicine vol. 14 124. 4 Apr. 2014
Reports including case-series studies		
Fujimori S.	High dose probiotic and prebiotic cotherapy for remission induction of active Crohn's disease	Journal of gastroenterology and hepatology vol. 22,8 (2007): 1199-204
Reports not accesible		
Malchow H. A.	Crohn's disease and Escherichia coli. A new approach in therapy to maintain remission of colonic Crohn's disease?	Journal of clinical gastroenterology, 25(4), 653–658, 1997
Prantera C.	Probiotics and Crohn's disease	Digestive and liver disease: Official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver vol. 34 Suppl 2 (2002): S66-7

Supplemental Table 2: Results of the included studies

Title/ Author (Year of Publication)	Study Design	Subjects with CD (sample size)	Disease activity score (baseline)	Probiotics studied	Duration of treatment	Placebo/other medication given	Effect	Results
Effect of mesalazine combined with probiotics on inflammation and immune function of patients with inflammatory bowel disease/ **Tan F. (2022)**	Retrospective cohort	28 (116)	CAI (not mentioned)	Bifida triple (Bifidobacterium longum, Lactobacillus acidophilus and Enterococcus faecalis)	2 months	Mesalazine alone	Total effective rate [(number of cases with markedly effective + cases of effective)/the total number of patients]. Effectiveness was measured based on the alleviation of symptoms and endoscopic healing.	Patients in the treatment group, which contained both CD *and* UC, presented with a 88.52% total effective rate vs. 70.91% in the control group.
A randomised, double-blind, placebo-controlled trial of a multistrain probiotic in patients with asymptomatic ulcerative colitis and Crohn's disease/ **Bjarnason I. (2019)**	Randomized, double-blind trial	62 (143)	Harvey Bradshaw (<5)	Symprove (Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus acidophilus, Enterococcus faecium)	4 weeks	Placebo		The total HBI score at the end of the study did not difffer between the 2 arms (Probiotics arm's score at the end: 3.5 ± 3.2 vs 3.4 ± 2.5 in the placebo arm, p= 0.66). The mean change was similar between the 2 groups: − 0.4 ± 2.2 in Symprove arm vs − 0.6 ± 1.9 in placebo arm, with the mean difference (95% CI) between probiotic vs placebo being 0.2 (− 1.0, 1.4). Among the participants, no disease relapse was recorded.

Title/ Author (Year of Publication)	Study Design	Subjects with CD (sample size)	Disease activity score (baseline)	Probiotics studied	Duration of treatment	Placebo/other medication given	Effect	Results
The probiotic VSL#3 has anti-inflammatory effects and could reduce endoscopic recurrence after surgery for Crohn's Disease/ **Fedorak RN (2015)**	Randomized, double-blind trial	120 (120)	CDAI (For VSL#3 169.7 +/- 83.1 and for placebo: 164.8 +/- 81.4)	VSL#3 consisting of 4 strains of Lactobacillus (L. pracasei, L. plantarum, L. acidophilus, L. delbrueckii), 3 strains of Bifidobacterium (B. longum, B. breve, B. infantis), and 1 strain of Streptococcus salivarius	Initially all patients received VSL#3 for 90 days, when they were reassessed. 81 patients continued to the open-label phase which would last for 9 months, with 56 patients completing the final endoscopic evaluation at day 365.	Placebo	Severe endoscopic recurrence rate (Rutgeerts' score 3 or 4)	At 3 months from the start of the trial, 9.3% (4/43) of VSL#3 group experienced disease recurrence versus 15.7% (8/51) in placebo group (*p=0.19*).
Saccharomyces boulardii in Maintenance Treatment of Crohn's Disease/ **Guslandi M. (2000)**	Randomized, open-label, controlled trial	32 (32)	CDAI (70 in S.B. And 68 in control)	Saccharomyces boulardii	6 months	Mesalamine alone	Risk of CD relapse	Relapse of CD was observed in 6.25% (1/16) of patients on treatment with mesalamine plus Saccharomyces boulardii versus in 37.5% (6/16) of patients in mesalamine alone (*p=0.04*).

Title/ Author (Year of Publication)	**Study Design**	**Subjects with CD (sample size)**	**Disease activity score (baseline)**	**Probiotics studied**	**Duration of treatment**	**Placebo/other medication given**	**Effect**	**Results**
Saccharomyces boulardii Does Not Prevent Relapse of Crohn's Disease/ **Bourreille A. (2013)**	Randomized, placebo-controlled trial	165 (165)	CDAI (71.4 in S.B. and 66.3 in placebo)	Saccharomyces boulardii	52 weeks	Placebo	Risk of CD relapse at week 52	38/80 (47.5%) in the S boulardii group and 42/80 (53.2%) in the placebo group had relapse at week 52 (*p=0.5*)-results were similar with the stratification factor (use of steroids or salicylates for induction of remission). After adjustment on the CDAI at randomization and on the stratification factor, the mean CDAI alterations from the beginning at the end point were 79.7 and 69.0 points in the treatment and control groups, respectively with the effect of the stratification factor not being statistically significant (*p=0 .99*).
Changes in the Crohn's Disease Activity Index and Safety of Administering Saccharomyces Boulardii in Patients with Crohn's Disease in Clinical Remission: A Single Hospital-based Retrospective Cohort Study/ **Oh GM (2020)**	Retrospective cohort	152 (152)	CDAI (40.65 ± 53.65)	Saccharomyces boulardii	6 months	No comparison with placebo	Reduction of CDAI	The total CDAI score decreased from 40.65 to 32.06 after 6 months (p<0.01).
Failure of Synbiotic 2000 to prevent postoperative recurrence of Crohn's disease/ **Chermesh I. (2006)**	Randomized, double-blind, placebo-controlled trial	30 (30)	Rutgreets score (surgery-not mentioned)	Synbiotic 2000, a commixture of probiotics and prebiotics, including 4 lactic acid bacteria (*1010 Pediacoccus pentoseceus, 1010 L. raffinolactis, 1010 L. paracasei susp paracasei 19, and 1010 L. plantarum 2362*) and 4 fermentable fibers (*2.5 g β-glucans, 2.5 g inulin, 2.5 g pectin, and 2.5 g resistant starch*).	24 months	Placebo	Post-surgical relapse rate	No difference was noted in either endoscopic or clinical relapse rate with the use Synbiotic 2000 or placebo-with Rutgeert's score being at month 3 0.8±1 in the placebo arm and 0.6±0.8 in the treatment arm-*p=not significant*.

Title/ Author (Year of Publication)	Study Design	Subjects with CD (sample size)	Disease activity score (baseline)	Probiotics studied	Duration of treatment	Placebo/other medication given	Effect	Results
Effects of pentasa-combined probiotics on the microflora structure and prognosis of patients with inflammatory bowel disease/ **Fan H. (2019)**	Randomized, placebo-controlled trial	9 (40)	CDAI	Bifico (Bifidobacterium, Lactobacillus acidophilus, and Enterococcus faecalis)	40 days	Mesalazine only	Change in CDAI score	The CDAI score, as well as the recurrence rate, in the observation group were significantly lower than those in the control group-3.86±2.16 in treatment arm vs 5.29±2.48 in control (all p<0.05). For the recurrence rate, only 1/ 21 (4.76%) in the treatment arm experienced recurrence versus 6/19 (31.58%) in the control group.
Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial/ **Marteau P. (2006)**	Randomized, double-blind, placebo-controlled trial	98 (98)	Rutgeerts score (surgery-not mentioned)	Lactobacillus johnsonii LA1	6 months	Placebo	Endoscopic recurrence rate	Endoscopic recurrence was observed in 27 of 43 patients (RR: 63%) in the placebo group versus 17 of 35 (RR: 49%) in the LA1 group (OR 1.79 (95% CI 0.72–4.42); *p=0.21*). **Severe** endoscopic recurrence was observed in 10 of 43 patients (RR: 23%) in the placebo group versus 7 of 35 (RR: 20%) in the LA1 group (OR 1.21 (95% CI 0.41–3.60); *p=0.73*).
Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG/**Prantera C. (2001)**	Randomized, double-blind, placebo-controlled trial	45 (45)	CDAI (surgery-not mentioned)	Lactobacillus GG	52 weeks	Placebo	Endoscopic recurrence rate	After 52 weeks of treatment, 15 patients (83.3%) treated with LGG and 17 (89.4%) treated with placebo remained in clinical remission (CDAI <150). Among patients remaining in clinical remission, nine of 15 allocated to the LGG group (60.0%) showed recurrent endoscopic lesions compared with six of 17 patients in the placebo group (35.3%) (p=0.297). Six of 15 patients who received LGG (40.0%) had severe endoscopic recurrence compared with three of 17 patients who received placebo (17.6%) (p=0.313)
Multicenter randomized-controlled clinical trial of probiotics (Lactobacillus johnsonii, LA1)	Randomized, double-blind, placebo-controlled trial	70 (70)	Rutgeert's score (i0)	Lactobacillus johnsonii(LA1)	12 weeks	Placebo	Difference in endoscopic score	Both in ITT and PP analysis and after 3 months of treatment, no significant difference in the mean endoscopic score was found between the two arms (LA1 versus placebo [n = 28 versus n = 27]: 1.50 ± 1.32 versus 1.22 ± 1.37, treatment effect: *p= 0.48*, smoke effect: *p= 0.72*). CDAI

Title/ Author (Year of Publication)	Study Design	Subjects with CD (sample size)	Disease activity score (baseline)	Probiotics studied	Duration of treatment	Placebo/other medication given	Effect	Results
on early endoscopic recurrence of Crohn's disease after lleocaecal resection/ **Van Gossum A. (2006)**								was not significantly modified after 4, 8 and 12 weeks of treatment (treatment effect: *p= 0.67*, visit effect *p= 0.004*; treatment and visit interaction: *p= 0.10*, mixed model) as it was not the mean histological score (LA1 versus placebo: 4.58 ± 2.82 versus 3.73 ± 2.19, treatment effect *p= 0.83*, mixed model after log-transformation) .

Supplemental Table 3: Results of studies on the Quality of Life and Inflammatory Markers

Title	Study Design	Subjects with CD (sample size)	Probiotics studied	Placebo/other medication given	Improvement in QoL	Changes in inflammatory markers
Effect of mesalazine combined with probiotics on inflammation and immune function of patients with inflammatory bowel disease/ **Tan F. (2022)**	Retrospective cohort	28 (116)	Bifida triple (Bifidobacterium longum, Lactobacillus acidophilus and Enterococcus faecalis)	Mesalazine alone	Not studied	In both groups, TNF-α, IL-6 and CRP decreased significantly ($p<0.05$) with greater reduction seen in the treatment arm ($p<0.05$)
A randomised, double-blind, placebo-controlled trial of a multi-strain probiotic in patients with asymptomatic ulcerative colitis and Crohn's disease/ **Bjarnason I. (2019)**	Randomized, double-blind trial	62 (143)	Symprove (Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus acidophilus, Enterococcus faecium)	Placebo	In both groups, the baseline QoL scores were similar and overall satisfactory. No statistically significant changes were seen after treatment in both groups	No statistically significant changes were seen in the evaluation of FCAL, CRP, ESR and WBC count
The probiotic VSL#3 has anti-inflammatory effects and could reduce endoscopic recurrence after surgery for Crohn's Disease/ **Fedorak RN (2015)**	Randomized, double-blind trial	120 (120)	VSL#3 consisting of 4 strains of Lactobacillus (L. pracasei, L. plantarum, L. acidophilus, L. delbrueckii), 3 strains of Bifidobacterium (B. longum, B. breve, B. infantis), and 1 strain of Streptococcus salivarius	Placebo	The IBDQ scores were similar in the 2 treatment groups (at3 and 12 months), without providing further information.	Not studied

Title	Study Design	Subjects with CD (sample size)	Probiotics studied	Placebo/other medication given	Improvement in QoL	Changes in inflammatory markers
Saccharomyces boulardii in Maintenance Treatment of Crohn's Disease/ **Guslandi M. (2000)**	Randomized, open-label, controlled trial	32 (32)	Saccharomyces boulardii	Mesalamine alone	Not studied	Not studied
Saccharomyces boulardii Does Not Prevent Relapse of Crohn's Disease/ **Bourreille A. (2013)**	Randomized, placebo-controlled trial	165 (165)	Saccharomyces boulardii	Placebo	Not studied	Changes in ESR and CRP among groups were similar and not statistically significant.
Changes in the Crohn's Disease Activity Index and Safety of Administering Saccharomyces Boulardii in Patients with Crohn's Disease in Clinical Remission: A Single Hospital-based Retrospective Cohort Study/ **Oh GM (2020)**	Retrospective cohort	152 (152)	Saccharomyces boulardii	No comparison with placebo	Not studied	An insignificant increase in ferritin [85.5 ng/mL (±85.3) to 87.7 ng/mL (±96.7), *p=0.62]*, CRP [0.24 mg/dl (±0.59) to 0.26 mg/dl (±0.463), *p=0.28*] and FCAL [217.4 µg/g (±382.4) to 233.1 µg/g (±378.7), p=0.64] levels were noted.

Title	Study Design	Subjects with CD (sample size)	Probiotics studied	Placebo/other medication given	Improvement in QoL	Changes in inflammatory markers
Failure of Synbiotic 2000 to prevent postoperative recurrence of Crohn's disease/ **Chermesh I. (2006)**	Randomized, double-blind, placebo-controlled trial	30 (30)	Synbiotic 2000, a commixture of probiotics and prebiotics, including 4 lactic acid bacteria (*1010 Pediacoccus pentoseceus, 1010 L. raffinolactis, 1010 L. paracasei susp paracasei 19, and 1010 L. plantarum 2362*) and 4 fermentable fibers (*2.5 g β-glucans, 2.5 g inulin, 2.5 g pectin, and 2.5 g resistant starch*).	Placebo	Not studied	A statistically significant decline in ESR was seen between the 2 arms after 3 months of follow-up, but not 2 years post-surgery-the decline was not statistically significant.
Effects of pentasa-combined probiotics on the microflora structure and prognosis of patients with inflammatory bowel disease/ **Fan H. (2019)**	Randomized, placebo-controlled trial	9 (40)	Bifico (Bifidobacterium, Lactobacillus acidophilus, and Enterococcus faecalis)	None	Not studied	After the intervention, the levels of hs-CRP, fecal lactoferrin and IL-6, which were similar at the beginning, were significantly lower, and the level of IL-4 was significantly higher in the observation group than in the control group ($p<0.05$).

Title	Study Design	Subjects with CD (sample size)	Probiotics studied	Placebo/other medication given	Improvement in QoL	Changes in inflammatory markers
Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial/ **Marteau P. (2006)**	Randomized, double-blind, placebo-controlled trial	98 (98)	Lactobacillus johnsonii LA1	Placebo	Not studied	Not studied
Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG/**Prantera C. (2001)**	Randomized, double-blind, placebo-controlled trial	45 (45)	Lactobacillus GG	Placebo	Not studied.	Not studied.
Multicenter randomized-controlled clinical trial of probiotics (Lactobacillus johnsonii, LA1) on early endoscopic recurrence of Crohn's disease after ileocecal resection/ **Van Gossum A. (2006)**	Randomized, double-blind, placebo-controlled trial	70 (70)	Lactobacillus johnsonii(LA1)	Placebo	Not studied.	The alterations in CRP values (3 months vs surgery) between both treatment groups were not significantly different (*p= 0.13*).

Abbreviations*: CRP: C-reactive protein, ESR: Erythrocyte Segmentation Rate, FCAL: Fecal Calprotectin, IL-6: Interleukin 6, IL-4: Interleukin 4, WBC: White blood cells*

Supplemental Table 4: Surgical interventions and precipitating disease behavior

Title	Study Design	Subjects with CD (sample size)	Probiotics studied	Type of surgery	Type of initial disease
The probiotic VSL#3 has anti-inflammatory effects and could reduce endoscopic recurrence after surgery for Crohn's Disease/ **Fedorak RN (2015)**	Randomized, double-blind trial	120 (120)	VSL#3 consisting of 4 strains of Lactobacillus (L. pracasei, L. plantarum, L. acidophilus, L. delbrueckii), 3 strains of Bifidobacterium (B. longum, B. breve, B. infantis), and 1 strain of Streptococcus salivarius	Ileocolonic	Not mentioned
Failure of Synbiotic 2000 to prevent postoperative recurrence of Crohn's disease/ **Chermesh I. (2006)**	Randomized, double-blind, placebo-controlled trial	30 (30)	Synbiotic 2000, a commixture of probiotics and prebiotics, including 4 lactic acid bacteria (*1010 Pediacoccus pentoseceus, 1010 L. raffinolactis, 1010 L. paracasei susp paracasei 19, and 1010 L. plantarum 2362*) and 4 fermentable fibers (*2.5 g β-glucans, 2.5 g inulin, 2.5 g pectin, and 2.5 g resistant starch*).	Not mentioned	Inflammatory (9/10 vs 18/20 in placebo and treatment groups), non-inflammatory (1/10 vs 2/20 in placebo and treatment groups)
Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial/ **Marteau P. (2006)**	Randomized, double-blind, placebo-controlled trial	98 (98)	Lactobacillus johnsonii LA1	Between LA1 and placebo: Ileal (13% vs 2%), Ileocolonic (83% vs 98%), Colonic (4% vs 0%)	Between intervention and placebo arms: Penetrating disease: 42% vs 50%

Title	Study Design	Subjects with CD (sample size)	Probiotics studied	Type of surgery	Type of initial disease
Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG/**Prantera C. (2001)**	Randomized, double-blind, placebo-controlled trial	45 (45)	Lactobacillus GG	Between intervention and placebo arms: Ileal: 69.6% vs 86.4%, Ileocolonic: 21.7% vs 9.1%, Colonic: 8.7% vs 1 4.5% ***Not statistically significant***	Between intervention and placebo arms: Obstructive (74% vs 68.2%), Penetrating (26% vs 18.2%) ***Not statistically significant***
Multicenter randomized-controlled clinical trial of probiotics (Lactobacillus johnsonii, LA1) on early endoscopic recurrence of Crohn's disease after lleo-caecal resection/ **Van Gossum A. (2006)**	Randomized, double-blind, placebo-controlled trial	70 (70)	Lactobacillus johnsonii(LA1)	Ileocaecal resection	Between intervention and placebo arms: Fibrostenotic (88% vs 86%), Perforating (22% vs 24%)

Supplemental Table 5: Evaluation of quality of the Randomised Trials based on the Risk of Bias 2 Assessment Form (RoB2)

	First author	Experimental	Comparator	Outcome	D1	D2	D3	D4	D5	Overall
Intention-to-treat	Bjarnason I.	Sympove	Placebo	Mean difference in HBI score before and after treatment in probiotic vs placebo arm	+	+	+	+	+	+
	Fedorak RN	VSL#3	Placebo	Severe endoscopic recurrence (Rutgeert's 3 or 4) at day 90	+	+	+	+	+	+
	Bourreille A.	S. Boulardii	Placebo	OR of CD relapse	+	+	+	+	+	+
	Chermesh I.	Synbiotic 2000	Placebo	Post-surgical recurrence of CD	+	+	!	+	+	!
	Fan H.	Bifico + Mesalazine	Mesalazine	CDAI score	+	!	+	+	!	!
	Marteau P.	LA1	Placebo	Endoscopic recurrence rate	+	+	+	+	+	+
	Van Gossum A.	Lactobacillus johnsonii(LA1)	Placebo	Difference in mean endoscopic score	+	+	+	+	+	+
Per-protocol	Guslandi M.	Saccharomyces boulardii + Mesalamine	Mesalamine	Risk of CD relapse	+	+	+	+	!	!
	Prantera C.	Lactobacillus GG	Placebo	Endoscopic recurrence rate	+	+	+	+	+	+
Appendix										
		D1: Randomisation Process **D2**: Deviations from intended interventions **D3**: Missing outcome data **D4**: Measurement of the outcome **D5**: Selection of results		+ Low risk ! Some concerns - High risk						

Supplemental Table 6: Evaluation of quality of the 2 Cohort Studies based on the Newcastle-Ottawa Scale

Cohort	Selection				Comparability	Outcome			Total
Study	**Representative-ness**	**Selection of non-exposed**	**Ascertain-ment of ex-posure**	**Outcome not pre-sent at start**	**On treatment ef-fect**	**Assess-ment of outcome**	**Sufficient follow-up**	**Adequacy (complete-ness of fol-low-up)**	
Tan F. (2022)	1	1	1	0	2	1	1	1	8
Oh GM (2020)	1	1	1	1	2	1	1	1	9