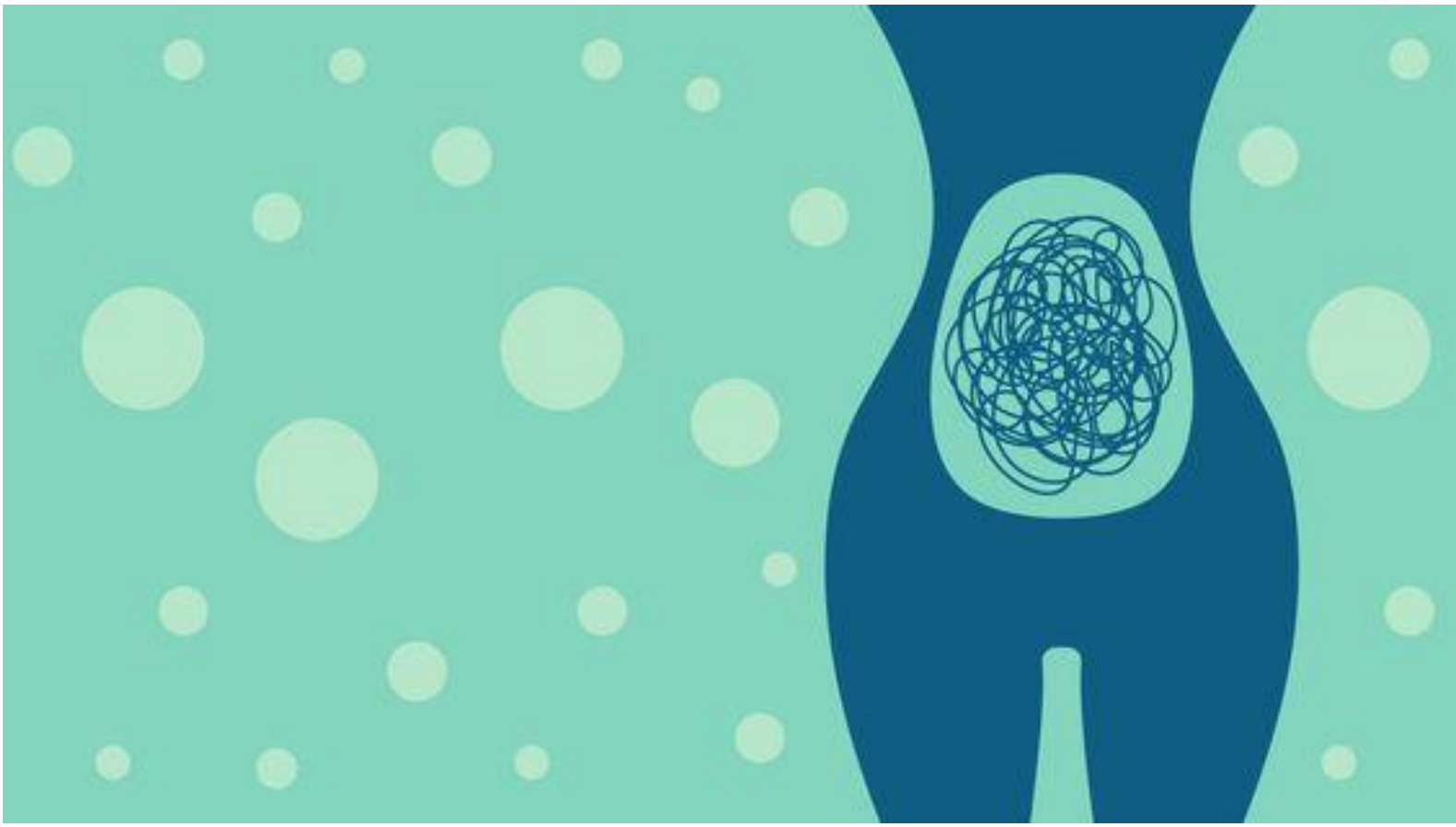

Differentiation between Irritable Bowel Syndrome from Inflammatory Bowel Disease flare-up through gut microbiota assessing test in quiescent IBD patients with IBS-like symptoms

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To my family, my hard work will be always dedicated to you. Thank you.

INDEX

1. ABBREVIATIONS

5-ASAs	Aminosalicylates
5-HT	Serotonin
Ab	Antibody
ADA	Adalimumab
AGE	Acute Gastroenteritis
ASCA	anti-*Saccharomyces cerevisiae* antibodies
ASUC	Acute Sever Ulcerative Colitis
AZA	Azathioprine
pANCA	perinuclear Antineutrophil Cytoplasmatic Antibody
CD	Crohn's Disease
CDAI	Crohn's Disease Activity Index
CNS	Central Nervous System
CPR	C-reactive protein
CT	Computed Tomography
CTE	Computed Tomography Enterography
EGD	Esophagogastroduodenoscopy
ESR	Erythrocyte sedimentation rate
FCS	Fibrocolonoscopy
FCP	Faecal calprotectin
GC	Glucocorticosteroid
GI	Gastrointestinal
GTP	Guanine nucleotides guanosine triphosphate
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
IBD	Inflammatory Bowel Disease
IBD-U	Inflammatory Bowel Disease Unclassified.
IBS	Irritable Bowel Syndrome
IC	Indeterminate Colitis

IFNγ	Gamma interferon
IFX	Infliximab
IL	Interleukin
IS	Immune System
JAK	Janus Kinase
MR	Magnetic Resonance
MRE	Magnetic Resonance Enterography
MTX	Methotrexate
NOD2	Nucleotide-binding Oligomerisation Domain protein 2
NPV	Negative Predictive Value
PPIs	Proton Pump Inhibitors
PPV	Positive Predictive Value
SBCE	Small Bowel Capsule Endoscopy
SCFA	Short Chain Fatty Acids
SI	Surgical intervention
SSRI	Selected Serotonin Reuptake Inhibitors
TCA	Tricyclic antidepressants
Th1	T-helper lymphocytes type 1
Th2	T-helper lymphocytes type 2
Th17	T-helper lymphocytes type 17
TLR	Toll-like Receptors
UC	Ulcerative colitis
UC-DAI	Ulcerative Colitis Disease Activity Index
US	Ultrasonography
USK	Ustekinumab
WCC	White blood cell count
WCE	Wireless capsule endoscopy

ABSTRACT

Background

IBD is a chronic relapsing disorder and represent a huge burden in the life of the individuals suffering from it. Moreover, once achieved the remission state, known as quiescent IBD, around one third of the patients will still present intestinal symptoms compatible with IBS (IBS-like symptoms). Even if both entities are well-defined and differentiate, in quiescent IBD patients with persistent clinic the diagnosis is not easy established through anamnesis and examination alone, and in most cases a colonoscopy is performed in order to confirm the origin of the symptoms. Therefore, there is a need for a non-invasive diagnostic test to differentiate between both entities without the performance of unnecessary colonoscopies. RAID-Dx is a new diagnostic tool based on the detection of the alteration on the main gut microbiota populations related to IBS and IBD from a stool sample. As IBS and IBD both have its own microbiological signature, this test has emerged as a potential, and inexpensive, alternative to colonoscopy in the assessment of IBS-like symptoms in patients with quiescent IBD.

Objectives

The main purpose of the study is to verify RAID-Dx test as an optimal diagnostic tool for the differentiation of IBS and IBD flare-up in patients with quiescent IBD suffering from IBS-like symptoms. For that reason, sensibility, specificity, PPV, PNV and likelihood-ratios will need to be established. Secondary objectives include the assessment of diet as an interaction factor of RAID-Dx results and the calculation of IBS prevalence in qIBD patients.

Design and Methods

This is a cross-sectional study performed in 6 different centres located in Girona, Figueres and Olot sanitary areas. The number of participants needed to recruit is 207 patients, from March to September 2020, and the sampling obtention follows a non-probabilistic consecutive design method. Bivariate analyses have been used to investigate potential associations between RAID-Dx results and various demographic, disease factors and diet habits.

Keywords: quiescent intestinal bowel disease; qIBD • IBS-like symptoms • Irritable bowel syndrome; IBS • RAID-Dx test • gut microbiota.

3.

INTRODUCTION

3.1 BACKGROUND

The need for a good diagnostic tool capable of differentiating IBS from IBD is increasing these days. Around 35% of the patients with quiescent IBD suffer from IBS-like symptoms, which is, they meet Rome IV criteria for this syndrome. Even though IBD and IBS could be considered two opposite extremes of the same functional-organic alterations (bacterial dysbiosis), the treatment differs drastically from one another, as well as the follow up and the implications of the disease. Nevertheless, there is not, nowadays, a good marker to differentiate both entities without the need for a colonoscopy (1). This could lead to a misdiagnosed relapse in a patient with quiescent-IBD and undergoing trough a unnecessary invasive procedures, receiving inappropriate treatment, as well as stress and psychological suffering for the person (2).

3.1.1. INFLAMMATORY BOWEL DISEASE

Concept and definitions

Inflammatory bowel disease (IBD) is a multifactorial, chronic and relapsing disorder of the gastrointestinal tract which includes three independent clinical forms under its name: ulcerative colitis (UC), Crohn's disease (CD) and indeterminate colitis (IC). The three entities share a main feature: the chronical inflammation in different areas of the intestinal tract without a well-defined aetiology. Although its physiopathology is not well understood, it has been associated with an immune dysregulation towards the gut microbiota in susceptible individuals (3).

Because of its clinic, radiologic and endoscopic heterogeneity, it's difficult to differentiate one disease from another. Therefore, we need to understand the conceptual differences between them.

Crohn's disease

CD is the extreme form of IBD. It can affect any part of the intestinal tract, from the oropharynx to the anus. The most frequent affected parts are the terminal ileum and the cecum. The isolated alteration of the rectum is not so common (more characteristic of UC) and, even less, the oropharynx affection.

One of its mains characteristics is the segmented inflammation, which is the alternation between healthy areas of gastrointestinal mucosa and affected ones, with a non-predictive extension and, also, asymmetry through the gastrointestinal tract. This inflammation is considered transmural which means the implication of the overall intestinal wall, from the superficial mucosa layer to the serosa, which covers the intestinal organs, including the mesenteric fat. These changes produce a characteristic effect of CD called *fat wrapping*, which is not found in UC (3).

Ulcerative colitis

We can define UC as a chronic inflammatory bowel disease which affects the colonic mucosae in a diffuse and continuous way. The most frequent presentation is the involvement of the rectum mucosa which progresses proximally towards de cecum. Once it reaches the ileocecal valve the wall alteration stops abruptly(3).

According to the extension of the disease, UC can be divided in 3 different types, with prognostic and treatment consequences:

- Spread colitis (20%): colonic alteration beyond splenic angle.
- Left colitis (30-40%): colonic affectation up to splenic angle.
- Ulcerous proctitis (30-40%): involvement limited to the rectum

Even though UC and CD are both included under IBD name, the management differ from one another, so the therapeutic options are not the same. This fact points out the need to establish a correct diagnostic between these two diseases, something not always as easy as it could seems.

The characteristically aspects of UC are the continuous affection of the colon and the sole involvement of this part, without the affectation of the hole gastrointestinal tract affectation as it happens in CD(3).

Histologically, the main characteristic of UC is the limitation of the alteration in the mucosa layer. There is a congestion of the lamina propria and an increase in plasmatic cells concentration, which usually associates with a crypt distortion. Moreover, another pathologic trait is the presence of a high number of neutrophils in the crypts walls (cryptitis), inside them (abscess) o associated with a destruction of the structure(3).

All of these changes are not pathognomonic, and the best histological factor related with UC is the assessment of chronicity: architecture deformity, crypt atrophy and the existence of a host of basal lymphocytes in the mucosa (3).

Indeterminate colitis

Indeterminate colitis is a chronic intestinal disease which affects, exclusively, the colon. What is needed in order to talk of IC?

- Colon alteration exclusively being rule out infectious causes and other potential causes of colitis.
- Mixt endoscopy lesions or not clear enough to make a diagnosis of neither UC nor CD.
- Crypt architecture unstructuring, more than 10%, with signs of acute and chronic inflammation in the histologic study.

In other words, it is a third form of IBD when neither the diagnosis of CD nor UC is clear. It seems to have a worst evolution than the other two entities and it does not respond as well as them to the treatment, specially to surgery (4).

Epidemiology

IBD are chronic diseases, which will represent a lifelong burden for the patient suffering from it, producing a significant impact in the quality of life of the person. One of the most preoccupying facts of these illnesses is the increasing in their incidence, not only on the West countries, which has been historically the areas where the disease was more spread out, but also on the East (Asian countries), where the diet factors were considered as protectors (5). Taking on account this last statement, is interesting the recent increase on the incidence of IBD in the East and South Asia, especially in UC and followed one or two decades later by CD, due to the changes in dietary habits in the population and the modification of microbiome that follows. Moreover, the incidence and prevalence of IBD varies within a country, where the individuals residing in areas with latitudes more distant from equator are at higher risk for IBD, perhaps justified by the difference between ultraviolet light exposure and vitamin D status as a modifier of the disease. In short, IBD, and its occurrence, is a dynamic process with an increasing incidence worldwide, particularly among the young ones, even in the areas previously considered in low risk. (6).

Even though the prevalence of IBD varies among one country an another, and also between the different forms of the disease, is estimated around 0'1% (7). Data suggested that the prevalence of IBD in United States is about 1.5 million individuals (440 cases per 100000 people), 2.2 millions in Europe (0.3% of the European population, particularly in Scandinavian countries and United Kingdom) and several thousand worldwide (6). Comparing the two main entities under the name IBD, UC and CD, one can see how the incidence and prevalence of UC are slightly higher than CD. Incidence ranges for UC vary between 0.9-24.3 per 100000 person-year, and for CD 0.5-10.6 cases per 100000 person-year. As for the prevalence, it differs from 2.4 to 294 cases per 100000 persons for UC and from 1.5 to 213 cases per 100000 persons for CD. The ranges are wide due to the difference on the presence of the disease according to the geographical area referred being, as said before, the highest in the countries of North-Europe (8)

IBD is a complex illness which usually has its onset in the childhood or early adulthood (peak age between second and third decades of life, mean age 30 and 35 for UC and CD, respectively) and keeps going the whole life of the person with a protracted course of remissions-relapses. It has been described a second, and smaller, peak of incidence between the sixth and seventh decades, especially in the case of UC, without a clear cause or factor explaining this phenomenon. (6).

Furthermore, as a chronic disease, IBD has also a great impact economically, with an estimated direct healthcare cost in Europe of 4.6-5.6 bn €/year and 1.2 billion $/year in Canada (8,9). Even more, not only the direct costs are alarming, but also the indirect ones associated with IBD, which refers to the expenses related with lost work productivity, disability coverage, premature retirement and death, estimated to be 1.5$/year just in Canada (10). As a matter of fact, underemployment and unemployment related to the intestinal disease has been reported in 10% and 8% of the patients respectively, especially among the ones suffering from CD(8). Apart from the economic costs itself, it is important to highlight the huge repercussions in the quality of life of the IBD patients, as well as their families, who may experience a reduction in school and work performance and social interactions and relationships (8).

As a matter of fact, without new discoveries that lead to a cure or prevention for IBD, the incidence and prevalence of the disease is expected to keep increasing rapidly in the following years, not only in the West countries but in every industrialised country all over the world. For that reason, there's a need for optimization the management of IBD and make a good use of the available resources, avoiding unnecessary tests and treatments, in order to be prepared for the rising burden of IBD (9).

IBD and colorectal cancer

The first data related to this topic was highly preoccupant. Due to a series of errors in the earliest studies about risk of cancer and mortality in IBD patients, the incidence of it was estimated to be falsely high. Luckily, the published results have been reviewed and the concept surrounding this topic has gain a more realistic interpretation (8,11).

IBD patients have an overall risk for CRC similar than the general population. Truth being told, a more accurate interpretation of that sentence needs to be done. Patients with CD present a risk for CRC close to the general population, whether in the ones with UC, especially the patients with more extensive disease, is twice as high as that of the general population, with an estimated absolute risk between 1-2'5% at 20 years, with a decrease in its ratio in the recent years (12). In UC patients, then, a colonoscopy is recommended after 8-10 years of the initial diagnosis. After that, further surveillance should be done according to physician criteria and patient preferences, being the usual behaviour the performance of a colonoscopy with 1-year to 2-year intervals (13).

Aetiopathogenesis

IBD represents a heterogenous disease without a clear and known aetiology, as well as its pathogenesis. Nevertheless, it is thought to be originated from a multidimensional pathway arrangement involving different elements and resulting in the appearance of the disease (14).

Even though its pathogenesis has not been yet well-understood, there are known triggers of the disease. One of the most important groups are the ones placed underneath the title "Western lifestyle factors" (see Annex 1). They are thought to produce a dysbiosis of gut microbiota and, in genetic predisposed individuals, to induce an abnormal response to the gut microbiologic population (15). Moreover, as these factors are related to IBD debut, and its relapses as well, they can even justify the rise of IBD incidence worldwide (16). The difference and impact of lifestyle factors and diet in IBD according to the geographical area is well-known, with countries in Asian-Pacific region considered as low-risk IBD areas. Although, as it is mentioned above, the changes in the dietary habits and lifestyle has promoted the increase of the disease even there.

Genetic influence

Genetic predisposition to IBD differs between CD and UC. Whereas it seems to play an important role in the first one (concordance of 40-50% in twin studies), it remains unclear its influence in UC (17,18). There have been assessed more than 160 risk alleles for CD, one of the most known ones being NOD2 the first gene discovered to be involved in CD (2001). Most of them are related to the interaction between bowel and intestinal immune system cells with gut microbiota (19). Another gens related to CD are: *ATG16L1* and *IRGM* autophagy-related genes and IL23R *loci*, among others (20).

Apart from the genetic influence itself, there is an increasing interest regarding the epigenetic factors in IBD, and the interplay between the host DNA and environmental and microbiological triggers, in order to explain the "disease memory" factor related, mostly, with CD (14). The main epigenetic modification which has been observed in IBD (mostly in studies of CD) is DNA methylation. It has been proposed the existence of specific methylation patterns for IBD that may justify the "epigenetic imprinting" related to IBD and the recurrence of the disease even after surgery (21).

Intestinal mucosal barrier alteration

When looking back in time, one can notice the change in tendency regarding IBD. First hypothesis considered the disease as an autoimmune-like one. Nevertheless, through the studies about the topic and the knowledge acquired this idea has been replaced for a complex pathogenic network involving different aspects of intestinal tract, such as an intestinal barrier disorder ("leaky barrier", Fig.1) and gut microbiota alterations (22).

Nevertheless, the barrier disorder seems to be different comparing CD and UC. In the first one the main problem could be an alteration of Paneth cells and a defect in defensin formation, due to predisposal genetic alterations and in bacterial recognition.

The inner mucosa layer, which should be sterile, is invaded by microorganisms, like adherent and invasive *Escherichia Coli*, and contribute to the inflammation process. On the other hand, UC has been associated with a disorder in the mucosal layer thickness, which is thinner than it should be (23,24).

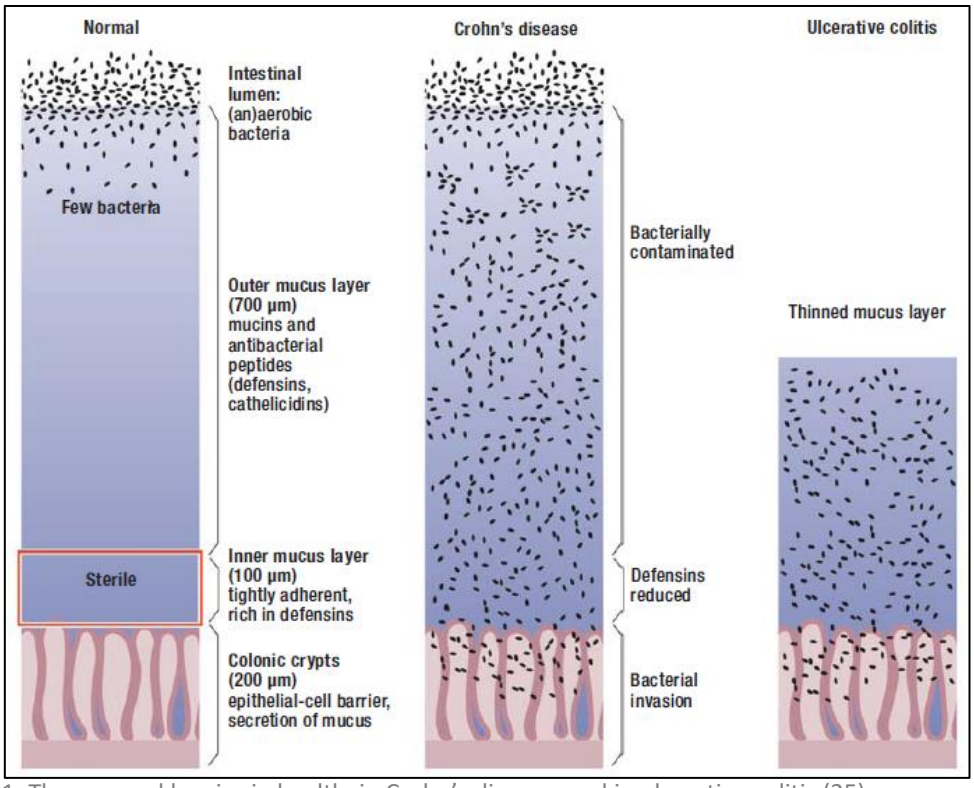


Figure 1: The mucosal barrier in health, in Crohn's disease, and in ulcerative colitis (25).

The cause behind the disruption in the intestinal barrier function is not fully understood. It has been hypothesised an interaction between environmental factors (western diet, gut microbiota dysbiosis and other environmental factors) over genetically sensible individuals. One of the genes proposed to be involved in this process is *Rho-A*, a family member of the RAS super family of small GTP-binding proteins involved in the cytoskeletal arrangement in epithelial cells. A deficient prenylation of the synthetized protein can help into the induction to IBD (26).

Gut microbiota in IBD

Through the study of microbiota changes in different communities, an approach of the bacterial profile of the disease has been obtained. The alteration in diet and immunity early in life, then the alteration of gut microbiome – immune system relationship, has been pointed out as one of the main factors for the development of IBD in the adult life (27,28). Nevertheless, the gut microbiome dysregulation in IBD is more complex, and it does not enrol only some specific genera, but different species and a loss of microbiota diversity, a fact assessed in various studies (29–32). Even more, bacteria are not the only microorganisms related to IBD, but fungus and viruses are implicated in its pathogenesis as well (33).

One of the biggest problems when assessing the microbiota is the lack of tools for doing that. Most of the studies related to gut microbiota and IBD use 16s-RNA gene-sequencing technique, including the test proposed in this study, which is based in the analysis of the 16s ribosome subunit of the bacteria, different from every specie and rather stable. Even though, this tool have allowed to do big progresses regarding gut microbiota in IBD, it only produce a crude estimation of the real changes in these patients, so new and better methods are required (34).

Immunological alterations in IBD

Even though IBD is not considered an autoimmune disease anymore, the role of immunity in its pathogenesis is clear. The current knowledge related to this topic is that an alteration in both, innate and adaptative, immunity systems are involved in IBD pathways (Figure 2). Moreover, it seems that the immune cells implicated in CD pathology are not the same ones as in UC. In the first one the main alteration is found in Th1 lymphocytes, whereas in the second one it has been implicated an aberrant Th2 response. Furthermore, Th17 have been related in of both entities (14).

Innate immunity alteration:

Microbial antigens from gut microbiota are recognised by TLRs in the dendritic cell surface and NOD-like receptors in the cytoplasm (mainly due to the rise in the intestinal permeability). The alteration on the expression and function of both receptors is the first step of the abnormal immune response in IBD. This fact linked with the defective epithelial barrier and increased intestinal permeability leads to the activation of the immune system and intestinal inflammation (20,34).

Adaptative immunity alteration:

The adaptative immune dysregulation begins with the differentiation of Th0 lymphocytes (naïve) through the anomalous presentation of commensal bacteria antigens as a threat by activated dendritic cells of intestinal mucosa. This lack of adaptation to the intestinal environment leads to the production of pro-inflammatory cytokines (especially IL-12, IL-23 and IFN-1) which stimulates the differentiation of the naïve lymphocytes to altered Th1 (IL-12 excess) and Th17 (IL-23 excess) cells and, consecutively, the loss of immune tolerance to commensal bacteria, with an imbalance between Th17 cells and Treg cells ratio (pro-inflammatory environment). The increase in Th1 cells is translated with IL-2, IL-12 and IFNγ, whereas Th17 is associated with excessive production of IL-17A, IL-17F, IL-21 and IL-22 (20,34,35).

The importance behind the understanding of the immunological pathways involved in IBD is the chance to develop new pharmacological targets for its treatment, such as the most recent ones anti-JAKs (tofacitinib). There still are a lot of challenges regarding IBD pathophysiology understanding, such as the roles of micro RNA (36) and inflammasome, the cause of genetic alterations, the cell sequence implicated in

it…Therefore, research about this topic needs to be done, in order to progress, as well, in the diagnosis and therapeutic management of the disease.

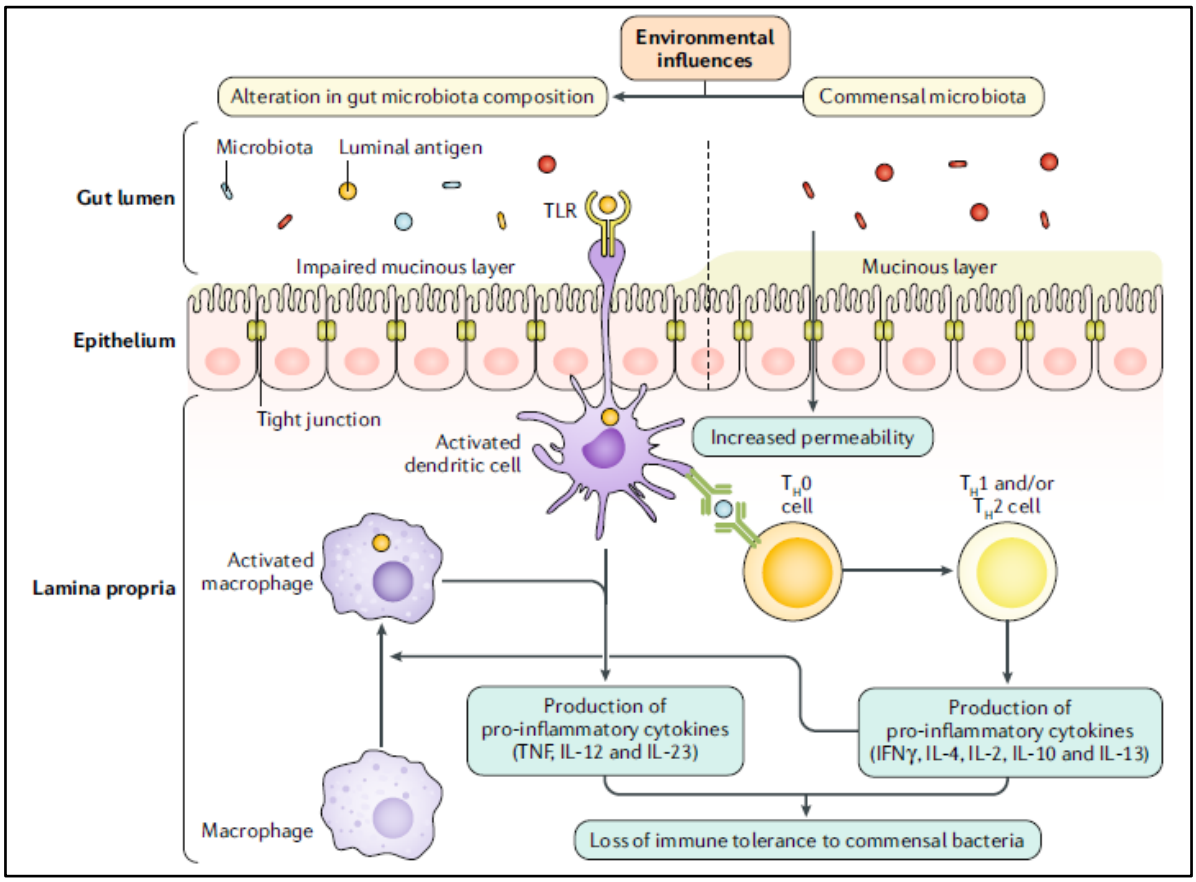


Figure 2: the hypothetical IBD pathophysiology (37).

Clinical manifestations

The signs and symptoms of IBD are extremely heterogeneous. The most frequent clinical manifestations which lead the patient to the medical consult, are (6):

- Diarrhoea, with or without blood.
- Abdominal pain.
- Haematochezia.
- Weight loss.
- Perianal affectation, such as abscesses, fistulas or fissures.

The course of the disease usually follows a flare-remission circle, but patients who respond to the treatment and remain stable are more prone to remain clinically inactive in the following year (13).

UC

Most patients report mild to moderate symptoms at the time of diagnosis, mainly as bowel rhythm alterations and haematochezia. The severity of the symptoms correlates with the extension of the disease, being pancolitis the maximum expression of UC.

Moreover, if the disease debut is linked to fever or weight loss, the risk of subsequent relapses increases.

CD

CD patients have more heterogenous symptoms. The initial clinical manifestations can be many and they are influenced by the level of affectation of the disease (3):

- Ileum: the symptoms are produced, mainly, by the production of stenosis at this level, with the resulting nausea, vomiting, abdominal pain, weight loss or fever.
- Colon: the symptoms resemble the ones mentioned in the UC, but usually more severe: diarrhoea (abundant), haematochezia, abdominal pain and weight loss. It is the phenotype which is associated the most with perianal disease and extraintestinal manifestations.
- Upper GI: the upper GI affectation presents highly heterogenous symptoms, which vary according to the affected intestinal portion: dyspepsia (nausea, vomits, epigastralgy, upper digestive bleeding) if oesophagus or stomach are affected or diarrhoea associated with weight loss and malabsorption syndrome if the affected zone is the jejunum (more frequent in the younger forms of CD).

Extraintestinal clinical manifestations

Both, UC and CD, are related with several extraintestinal symptoms, some of them more specific of one entity or another. This happens in 25%-40% of IBD patients. Almost any organ or system can be affected by IBD, but the more frequent symptoms are the ones involving the eyes (episcleritis), skin (erythema nodosum), joints (the most frequent one, especially peripheral arthritis) and liver (lithiasis, sclerosing cholangitis ad steatosis). The first three manifestations are more common in colonic IBDs whether the last one is more characteristic of CD as a direct extension of the disease. Most of them follow the course of the disease and respond to its treatment (38).

In order to make this introduction section more pleasant, the reader is referred to the Annex 2, if the topic is of their interest (39,40).

Classification of IBD

The classification within IBD are used in order to guide the better treatment for the person and to be able to assist with service delivery and research (41).

Ulcerative colitis phenotypic classification

There are two main phenotypic classification for UC: Montreal classification (Table 1), for adults, and Paris classifications, used in children.

Table 1: Montreal UC Classification for adults. Adapted from: (42).

MONTERAL UC CLASSIFICATION	
Extent (maximal macroscopic inflammation)	E1: ulcerative proctitis
	E2: left-sided UC (distal to splenic flexure)
	E3: extensive (proximal to splenic flexure)
Severity (assess with UCAI)	S0: clinical remission
	S1: mild UC
	S2: moderate UC
	S3: severe UC

It is important to keep in mind these classifications are not static, and even if a patient has been labelled with a concrete phenotype the extensions and severity of the disease can change. This can be seen even in a half of the persons with initially proctitis or proctosigmoiditis who end developing a more extensive affectation (43). Nevertheless, this fact does not apply otherwise. If the extent of the inflammation would regress over the time (generally associated to an optimal therapeutic response) the classification was to be kept to the maximal extent the disease got (4)**.**

Moreover, the different phenotypes of IBD have its own implications. In the case of the extensive colitis, for example, it has been reported a high risk of developing colorectal cancer (44).

Crohn's Disease phenotypic classification

CD phenotype is defined using Montreal classification (Table 2), which divides the patients according to age, localization and extent and behaviour of the disease. These three parameters have been recognised as the main ones influencing the course and prognosis of the disease. Moreover, phenotypic concordance in CD (as well as in UC) has been reported in familial cases of IBD and in monozygotic twin studies (4,45).

Table 2: Montreal CD Classification for adults. Adapted from: (42)

MONTREAL CD CLASSIFICATION	
Age (A)	A1: ≤16 years
	A2: 17 – 40 years
	A3: >40 years
Localization and extension (L)*	L1: ileal (terminal ileum)
	L2: colonic
	L3: ileocolonic
	L4: isolated upper GI disease
Behaviour (B)†	B1: non-stricturing; non-penetrating (superficial ulcers)
	B2: stricturing (with dilation of the pre-stricturing portion)
	B3: penetrating or fistulizing

*Upper GI modifier: (+ L4) is a modifier that can be added to L1-L3 when concomitant upper-gastrointestinal disease is present.
†*p* perianal modifier: "p" is added to B1-B3 when concomitant perianal (mainly fistulizing) disease is present.

Ulcerative colitis and Crohn disease activity assessment: Mayo score and SES-CD

Apart from the phenotypic classification of IBD, there are also endoscopic scores in order to assess the inflammatory state of the intestinal mucosa. By doing so, one can assess the severity of the disease and decide the most appropriate therapeutic management according to it. There are different endoscopic classifications available, ones more intuitive while others more difficult to apply. The main one used in UC is Mayo score (Annex 3), whereas for CD is SES-CD (Annex 4).

Diagnosis

The main key for the diagnosis of IBD is clinical suspicion. Due to its unclear aetiology, multiple, but unspecific, risk factors and the various forms of clinical presentation, IBD is an entity to take it on account when making a differential diagnosis of chronic diarrhoea or constipation, associated, or not, to abdominal pain (6).

A single gold standard for the diagnosis of IBD is not available, to confirm the disease it requires a combination of the clinical evaluation, laboratory tests, radiological investigation (imaging), endoscopy findings and histological analysis(46).

Medical history and examination

The first step, then, is the clinical suspicion. Sometimes the signs and symptoms are highly suggestive of IBD, such as with UC (proctocolitis), but there are other times when this is not as clear as it could seems. The most frequent trigger symptoms have been stablished above (6), being the most common presenting symptom chronic diarrhoea, defined as a decrease in faecal consistency, or more than three defecations per day, for more than 6 weeks, even though it is a highly unspecific symptom(46). Nevertheless, as mention before, the clinical presentation can vary, particularly with CD. When affecting the upper gastrointestinal, for example, the presentation predominant symptoms are nausea, vomiting, epigastric pain and dyspepsia, usually associated with a decline of the general condition (weight loss, anaemia or fever)(6).

Furthermore, it is important to have in mind the extra-intestinal manifestations, common in patients suffering from IBD (Annex 2). The general idea is that any system can be affected, for that reason a detailed examination is required (47).

The patients' clinical history, when suspecting IBD, must include: detailed questioning about onset of the symptoms, recent travels, food intolerances and allergies, medication use, particularly antibiotics and non-steroidal anti-inflammatory, presence of known risk factors, family history and surgery antecedents (especially appendicectomy)(46). As mentioned before, one also need to carefully examine the person general well-being, cardiovascular function (pulse rate, blood pleasure), temperature, abdominal features (auscultation, abdominal tenderness or distension, palpable masses), perineal and oral inspection and digital rectal examination (46).

Laboratory testing

The second step for IBD diagnosis is a laboratory investigation, not as a diagnostic tool *per se* but a supportive valuable test to assess the patient condition. In this first analytical approach one needs to determine signs of acute or chronic inflammatory response, full blood count, fluid depletion and patients' nutritional status.

CPR is the inflammatory marker par excellence, nevertheless, its diagnostic usefulness is limited. Even though it has a high sensitivity, particularly for CD (in UC is around 60%), its specificity is low, and it can be influenced by other conditions, such as an infection (6). The other wide-use inflammatory marker is ESR, but it does not correlate so well with neither CD (better with colonic rather than ileal disease) or UC (46).

Other common analytical changes are anaemia and thrombocytosis, in the full blood count, and it is not strange to observe a vitamin o mineral deficiency, especially in patient with CD (46).

Apart from a blood test, stool testing for *Clostridium difficile* toxin and stool culture should be performed in order to rule out an infectious colitis. This entity can mimic IBD symptoms and, besides, it has been implicated as a precipitating factor for IBD. An active infection does not exclude the option of a concomitant IBD, so the diagnostic study must carry on (6).

Endoscopy

Endoscopy techniques represent the mandatory test for the diagnosis of IBD and one of the first-line diagnostic modalities when IBD is suspected. Colonoscopy is always performed and, according to the clinical suspicion, an esophagogastroduodenoscopy is also required (CD). When performing the colonoscopy, the examination must include the terminal ileum, one of the main affected zones in CD (ileocecal area) (6).

The endoscopic exam should include the assess of the macroscopic appearance of the colonic and ileum mucosa and, as well, the obtention of patched and random biopsies from these zones, even if the macroscopic examination does not reveal any remarkable alteration (46).

Even if UC and CD are entities under the same name, the endoscopic and histopathological findings are usually different between each other.

In CD, the most useful and definitory endoscopic features include (6):

- Discontinuous and skipping involvement.
- Cobble stoning.
- Anal lesions.
- Ileal inflammation.
- Fat wrapping, which refers to the hypertrophy of the surrounding fat and its envelopment of the intestinal affected area.
- Aphthous and/or serpiginous ulcers.

- Rectal sparing.

As for the UC concerns (6):

- Continuous and circumferential inflammation, particularly proximal to the anal canal.
- Granularity.
- Loss of the normal vascular pattern.
- Friability.
- Spontaneous bleeding.
- Pseudopolyps.
- Superficial ulcerations.
- Abrupt transition between healthy and diseased mucosa.

Ileocolonoscopic has been proved to be the best diagnostic modality in order to assess IBD and differentiate between the two entities, UC and CD, with an accuracy around 90%, better than imaging techniques (6)(46). However, it is true that a few percentages of cases can have a non-definitory FCS, due to some findings which can confound the diagnosis. These overlapping alterations include (6):

- Backwash ileitis present in about 25% of cases of extensive UC.
- Caecal affectation or periappendiceal patch.
- 54% of the patients with small-bowel CD have a normal ileoscopy.

As mentioned above, it is not enough the visualization of the macroscopic appearance of the bowel, it is also required a microscopic analysis through the biopsy samples obtained (see below). Nevertheless, it is important to highlight that there are no definitory histological criterion able to conclusively establish a diagnosis of IBD (6).

A part from the definitory findings for each IBD entity, endoscopy helps to stablish anatomical criteria of severity (46):

- Deep ulcerations eroding the muscular layer.
- Mucosal detachment.
- Ulcerations limited to the submucosa but extending to more than one third of a defined colonic segment, which are right, transvers, left and sigma-rectum.

When these alterations are present, the risk of bowel perforation due to the test is high, so the FCS is usually postponed until the activity of the disease diminish.

Imaging

Imaging techniques are complementary to endoscopy. Its main function is to establish the extent of the disease, as well as the possible complications and the existence of penetrating lesions, particularly in CD. Furthermore, imaging techniques can help in the assessment of IBD activity and the detection of extramural complications (abscess, fistulae and inflammatory conglomerates) (46). As a matter of fact, there is a described image in MRE in the presence of a fistula formation called *the star-sign*.

The chosen radiological exams, with the highest diagnostic accuracy for the detection of intestinal involvement and penetrating lesions, are MRE and CTE, with a similar efficacy. Although, if MR is available should be used rather than CT, because the radiation exposure resulting from it and the need to repeat these examinations over time, generally in young patients (46).

MR and CT with oral luminal contrast, and intravenous contrast as well, are the best options when the small bowel needs to be assessed. Both techniques are capable to demonstrate disease extension and activity, based on wall thickness and the increased of intravenous contrast enhancement (46). The luminal inflammation is presented as:

- Bowel-wall hyperenhancement.
- Bowel-wall thickening.
- The *comb sign*, due to the engorged vas recta.
- Fibro-stenotic strictures.

For that reason, when facing a diagnosis of IBD an MRE, if not available a CTE, must be performed in order to stablish the extent of the disease and to assess the existence of complications and disease activity.

IBD serologies

There have been studies defending the detection of autoantibodies as an additional diagnostic tool for IBD. These proteins include pANCA, especially in UC, and ASCA, elevated mainly in CD. Nevertheless, there is a lack of evidence respect its sensitivity and specificity and, therefore, are not recommended in the routine screening of IBD (6).

Even though, it is true these autoantibodies may be helpful in the classification of IC between CD (ASCA+), CD (pANCA+) and UC (pANCA+) (6). High levels of ASCA in CD patients are related to a more aggressive phenotype, translated as an early onset of the disease with a penetrating/fibrostenosing behaviour, whereas pANCA in CD have been linked to a less severe form of CD with a UC-like behaviour and minor risk of small-bowel complications. As for pANCA in UC, they have been related with a more severe form of disease and higher needs of surgery (48,49).

Moreover, ASCA has been seen to remain in high concentrations in plasma even with the disease in a quiescent CD, leading to new lines of investigation in the use of autoantibodies as predictors of relapsing CD or potential complications(50). Not only this, but the levels of ASCA are related, as well, to an early need of anti-TNF drugs, so as higher they are the earliest anti-TNF treatment will be needed (50). Whereas this information is enough to justify its use in the clinical practice is up to future studies about this topic and the demonstration that an early treatment with anti-TNF improves the future response of the patient and reduce the failure rates of the treatment.

Faecal biomarkers

Faecal markers of inflammation have been shown to correlate well with the presence of intestinal inflammation. The most studied and known one is the faecal calprotectin

followed by lactoferrin (46). The first one is a calcium and zinc-binding protein present in the cytosol of neutrophils (represents the 60% of total cytosol proteins) and is released during apoptotic processes and necrosis remaining stable in the faeces for 1 week. Therefore, its faecal concentration correlates with the neutrophilic influx into the intestinal tract, a feature of active IBD (51). On the other hand, there is lactoferrin, an iron-binding glycoprotein of the neutrophils (secondary granules) which is released during the acute inflammatory response due to neutrophilic degranulation and adhesion to the endothelium. As one can see, then, lactoferrin plays the same role as calprotectin, an indicator of neutrophilic influx. Nevertheless, is less stable than the previous one.

Both biomarkers are significantly and consistently increased in active IBD (6). Although, one of its main problems is the establishment of a cut-off value. A low value is useful in order to exclude, with confidence, the patients without active intestinal inflammation, which is a high specificity, but giving up on sensitivity. Otherwise, a high value will provide an increased sensibility but will diminish the specificity (52).

One should take in account that these markers have been tested and studied especially in the prediction of IBD relapse more than in the initial diagnosis approach (46). Although, the results of the studies proving the accuracy of faecal biomarkers in the relapsing IBD have been extrapolated to the use in the first diagnostic approach of IBD and to assess organic origin in patients with GI symptoms, and the consequent need of further endoscopic evaluation (6).

The current cut-off levels for each marker are 50µg/g stool for calprotectin and 7.25µg/g stool for lactoferrin. The main uses of the first one are the follow-up and monitorization of patients in relapse, to differentiate the patients with IBD from the ones without inflammation (initial approach), while lactoferrin has proved to correlate well with clinical symptoms and endoscopic inflammation (6).

An important consideration about FCP is its predicting value variation according to the localization of the intestinal alteration and the mucosa healing ratio reached. Then, FCP has been shown to be a better predictor of relapse in UC and colonic CD, than ileal CD in which the sensibility and specificity dropped considerably. Furthermore, is better as a biomarker in UC than in CD, because the mucosa healing rate is higher in the first one (more than half of UC in remission comparing with 10% in CD), so the changes induced by a relapse (inflammation) produced a greater alteration (51).

Even though FCP and lactoferrin are the two main faecal biomarkers, other ones have been studied. Although, its prognostic value, as well as its use in monitorization, is still unclear. From the different ones assessed, it is worth mention S100A12, a S100 protein like calprotectin with a potent chemotactic activity and upregulated by TNF-α. It can be measure in serum or faeces and its value can reflect the presence and severity of intestinal inflammation. However, further investigations are needed.

Histological findings

In order to get a reliable diagnosis of IBD. It is required, at least, two biopsy specimens for each side from five sites of the colonic tract, in which must be included the ileum and rectum (53). The areas from where the samples are collected should mixed macroscopic involved mucosa zones and uninvolved ones (46). Once obtained, tissue samples should be fixed immediately by immersion in buffer formalin (or equivalent), then transported.

There is no pathognomonic histologic finding or alteration that confirm UC diagnosis. The suggestive features are: basal plasmacytosis, diffuses crypt atrophy and distortion, villous surface irregularity and mucous depletion (54). As for CD, the characteristic microscopic features are: focal or patchy transmural inflammation, aggregated inflammatory pattern and transmural lymphoid hyperplasia, crypt irregularity, submucosal thickening (fibrosis-fibromuscular obliteration), fissures, sarcoid granuloma, abnormalities in the enteric nervous system and goblet cells often spared, patched or discontinued lesion distribution with apparently healthy interlesional mucosae and variable size ulcers ("hole puncher" image) (3,46).

Other diagnostic techniques

Transabdominal ultrasonography: it is a well-tolerated imaging technique, non-invasive nor irradiating. It is used in CD, mainly, because of its limitation assessing the hole colonic extension (specially the transvers colon), in order to detect disease activity (increased bowel thickness) in the regions where the ultrasounds can reach (ileocecal, sigmoid and, in some cases, ascending and descending colon). Its sensitivity and specificity are improved with the use of contrast-enhancing abdominal US and Doppler US (55).

Small bowel capsule endoscopy or WCE: it is a diagnostic tool which allows a direct visualization of small bowel; therefore, it is used to rule out CD affectation in this region. Its sensitive and NVP are high allowing small bowel disease if its normal, unfortunately its lacks specificity. The current recommendation for its use is in selected-CD patients with symptoms suggesting small bowel affectation plus either extraintestinal manifestations associated, high inflammatory markers and/or abnormal small-bowel imaging. It is contraindicated in patients with suspected intestinal stricture, due to the risk of capsule retention which may require surgical removal (6,55).

Gastroscopy: it will be performed in patients with CD suspicion and upper GI tract symptoms, as well as part of the study of indeterminate IBD. There is no consensus whether upper GI endoscopy should be performed in asymptomatic adult patients or not (55).

Therapeutic management

The therapeutic management of IBD patients is a huge challenge for the clinician. There is no treatment available to cure the disease itself, and the medical and surgical strategies are used to delay the progression of the disease and prevent from the chronical inflammation from IBD.

The initial approach, into the therapeutic options one can offer to a person suffering from the disease, is to perform an estimation of the severity of their IBD. Once known that fact, the clinician will choose the optimal therapy for the patient according to different items that will define the severity of the disease (6).

One of the main problems is the lack of strong severity scales and scoring systems able to define with high accuracy the real situation of the disease. The reason behind this is in the items used in these different scores: symptoms, endoscopic criteria, histology, impact of the disease in the quality of life and the risk of progression and complications. All of them are useful tools to assess IBD but not always correlate between each other. For that reason, the division from the severity scales used in patients with IBD, mild, moderate, sever and fulminant, is not exclusive and, sometimes, there are components of different categories in the same patient (6).

The appropriate management of IBD requires, then, an assessment of the inflammatory condition of the disease, which is, an objective evidence of active inflammation or not. In order to achieve that, the clinician needs to evaluate the clinics, laboratory data and endoscopic findings. The last aspect is the most important one, because it is the most reliable in order to determine the presence of inflammation due to the IBD. As mentioned before, there are two different indices to assess IBD endoscopically, SES-CD for CD and Mayo score for UC (6).

Medical management: options in IBD

The first statement that needs to be clarify is there is no cure for IBD. Once the person is diagnosed with the disease will carry it permanently. For that reason, the aim of the therapeutic measures must be to minimize the symptoms, improve the quality of life, stop the progression of the disease and reduce the complications associated to it (13).

There are multiple medical options for IBD treatment. Therefore, that does not reflect a wider range of therapeutic options, as it could seem, but the difficult in its management and the necessity to perform a stagger treatment strategy (6). The treatment must always be based on the localization, extension and course of the disease, as well as individual factors and the clinical response of the patient. Moreover, most of the drugs used in IBD have important side effects one must be aware of it and implies the need to control their appearance and changing the strategy according to it.

- **5-ASA drugs**: the first ones used in UC, sometimes in CD (L2). They can be administrated either orally or by rectal enema (topically) and can be used as induction and/or maintenance treatment.

Even though these drugs are well-tolerated drugs, the main adverse effect related to 5-ASA treatment is its nephrotoxicity. It has been estimated at 1 in 4000 persons per year, with long-term 5-ASA therapy without a clear cause justifying it, such as tubulointersticial nephritis (56,57). For that reason, it is recommended the monitorization of baseline renal function in IBD patients treated with 5-ASA at the starting of the treatment, after 2-3 months and, then, annually (58).

- **Corticosteroids**: one of the most effective drugs used in IBD. Nevertheless, due to their side effects, GC are only used as induction therapy in active IBD. The administration of the GC can be orally (prednisolone) or topically, diminishing the systemic effects of the drug (not avoiding, well it is also absorbed) (58). The recommended topic GC are budesonide MMX and beclomethasone dipropionate (59,60). In case of facing a moderate - sever IBD flare-up, the recommendation is the use of oral prednisolone 40mg daily during 6-8 weeks, followed by a de-escalation instruction (58). If there is no clinical or endoscopic response in 2 weeks the treatment escalation to biologics, as well as the assessment of hospitalization need, must be considered (58,61).
- **Thiopurines**: particularly azathioprine. It has shown a benefit in the maintenance of induced remission, but not for the induction of remission itself. Even though they have an important number of secondary effects most of the patients tolerate them well, nevertheless one must be careful in the older patients taking this drugs, especially (62).
- **Methotrexate**: this anti-folic acid drug is used as a second line when thiopurines are not effective in CD (not in UC). One of its main side effects is hepatotoxicity and follow ups must be done in patients consuming it. Others include narrow suppression and pneumonitis.
- **Anti-TNF drugs:** monoclonal autoantibodies against the α-TNF. These drugs are used in the maintenance treatment of IBD. There are different options: infliximab, adalimumab and golimumab, are the three first ones to be used. There is not enough strong evidence to suggested which one is better, but it would seem that its efficacy is similar (63,64). One of the main factors in order to choose one treatment or other is the patient choice. IFX is administrated intravenously every 4-5 weeks, so the patient has to come to the hospital in order to get the treatment, and ADA can be injected subcutaneously at home. To have in mind, when an anti-TNF agent does not success in the resolution of the disease and there is a need to use another drug of the same family, this change is called a “switch”, whereas if a drug with a different mechanism (another family) is used the term used is “swap”.

  The anti-TNF therapy is usually combined with and immunosuppressor treatment (AZA) with the goal to reduce the organism immunologic response against the monoclonal antibodies and extend its efficacy and response ratio.
- **Vedolizumab**: one of the most recent developed drugs, therefore the one with there are less information about. It is an anti-integrin$\alpha_4\beta_7$. The administration is intravenously and its efficacy in achieving clinical and endoscopic remission

(asses with Mayo scores) seems to be superior to adalimumab in two recent studies (65,66). Nevertheless, until today it is recommended in the induction and maintenance of remission of UC when the other anti-TNF drugs are no longer effective (58).

- **Natalizumab**: an anti-IgG4 used in the induction and maintenance of CD.
- **Ustekinumab**: is an IgG1 monoclonal antibody which main function is to antagonize the biologic activity of IL-12 and IL-23, interacting in the binding process of this proteins into their receptors on the T lymphocytes. Its use has only been approved for CD (58).
- **Tofacitinib**: is an oral JAK inhibitor (JAK 1 and 3, and weaklier JAK 2). Its pathologic foundation is the interaction in the activation, proliferation and function due to the antagonizing effect with different ILs that take part in these processes. It has been approved in the UC treatment (moderate-sever) when the other available therapies fail. Between its side effects it is worth mention the haematopoietic alterations and the increase of herpes zoster infection compare to placebo, and zoster vaccination should be considered before the starting of tofacitinib treatment, especially in patient over 70-year-old (at least 4 weeks before the starting of the treatment) (67,68). Furthermore, it seems to there is a high risk of thrombotic events, particularly pulmonary thromboembolism, in the patients who use tofacitinib, although there is no enough data about this topic (69).

Ulcerative colitis management

This part is going to focus its attention in the management of UC. One must differentiate two main scenarios: on one hand the patient with an acute situation because of a high activity of its disease (flare-up) and, on the other hand, the patient with a known UC who requires treatment in order to control the symptoms and the chronical inflammation (see Figures 3-5).

Firstly, the management approach in recently diagnose UC is going to be discussed.

The initial treatment of an active UC is based on 5-ASA drugs, the most used one being mesalazine. This is the first option when facing a mild-moderate form of the disease. The doses varies among 2-3g one per day orally (58,70–72). Nevertheless, it seems that a dose of 4.8g/day is associated with a quicker symptom resolution ad a higher rate of mucosal healing at 3-6 weeks (73,74). If there is no response in 8 weeks high dose 5-ASA is recommended, as well as the addition of a 5-ASA enema (topical therapy) (58). Even more, all patient with UC should be offer with a combination of 5-ASA, orally and by enema, because of the higher efficacy of oral and topical treatment compare to monotherapy (oral) (75,76). However, the enema formula lacks acceptation among the patients and, even with the benefits that has shown, the difficulty of its administration and retention is a big obstacle for its use (58). To sum up, the actual recommendation is to start with a 2-3g/day of 5-ASA associated, if it is possible, topical treatment and, if there is no response, increase the dose up to 4-4.8g/day orally plus enemas (58).

The second line therapy are corticosteroids. This drugs are more effective than 5-ASA, nevertheless, their side-effects are high and they are reserved in case of failure (77).

Once treated the active UC, the maintenance treatment recommended is 5-ASA at 2g/day orally or rectally (in case of distal UC) (78). Although, what options are left if 5-ASA treatment fails? The option of GC as a chronic therapy is not the ideal choice because of its high side-effects and should be avoided. Therefore, in that point is when the IBD therapeutic pyramid starts to build up.

The next therapeutic drugs available are the thiopurines. When one can consider the right moment to start another treatment? According to the last British guidelines on the management of IBD, when high-dose of mesalazine are required and, even though, patients also need two or more courses of GC in the past year, or the ones who become corticosteroid-dependent, treatment escalation is recommended with thiopurine or anti-TNF therapy according to clinical factors, patient choice, cost, likely adherence and local infusion capacity (58).

If AZA is not well-tolerated or its efficacy is low, it is time to move to the next step: the biologic drugs. It is important to mention that even in CD the alternative to AZA is the methotrexate, in UC this drug has not proved its efficacy to placebo. Therefore, MTX has no role in its maintenance treatment (79,80).

The treatment with anti-TNF agents is the next option in the management of UC in remission. Nevertheless, before starting an anti-TNF therapy, there are some considerations to take on account. Any patients who is going to receive a biologic therapy must go through a previous evaluation in order to assess and prevent potential risk related to the treatment. This evaluation must include and discharge (81):

- TBC infection, which means to perform a Mantoux test and/or *interferon-γ release assay* (IGRA) plus a chest X-ray. If it exists an active infection the patient must receive the optimal treatment before the start of the biologic agent.
  Active hepatic infection must be rule out as well through HBV and HCV serologies.
- Diffuse interstitial Lung Disease, by a chest X-ray and an accurate anamnesis.
- Cancer, if the patient has a history of limproliferative neoplasia biologic treatment must be reconsidered.
- Hepatic diseases, by a blood test with hepatic profile and function parameters.
- Cytopenia, by a blood test with haematologic parameters.
- High cardiovascular risk. It is recommended the assessment of cardiovascular risk of the patient before the start of the treatment with biologic drugs, as well as the existence of heart failure.
- Demyelinating disease, such as multiple sclerosis.
- Vaccination: anti-pneumococcal and anti-influenza inactive vaccines must be provided before the start of the treatment. Consider human papilloma and hepatitis A vaccines, as well as HBV vaccine in patients who did not have receive

it previously. Live-attenuated vaccines are contraindicated once started the administration of the biological drug.

In case of anti-TNF treatment failure after switching to different drugs of the family, the next option recommended in the guidelines is vedolizumab (58).

Finally, in case of no response to neither of these therapies, last option for UC are ustekinumab and tofacinib. Regarding ustekinumab, even though it may be useful in case of failure of the other therapies its use in UC has not been approved yet, unlike in CD (58). Tofacitinib, it is the final therapeutic strategy when other ones have failed.

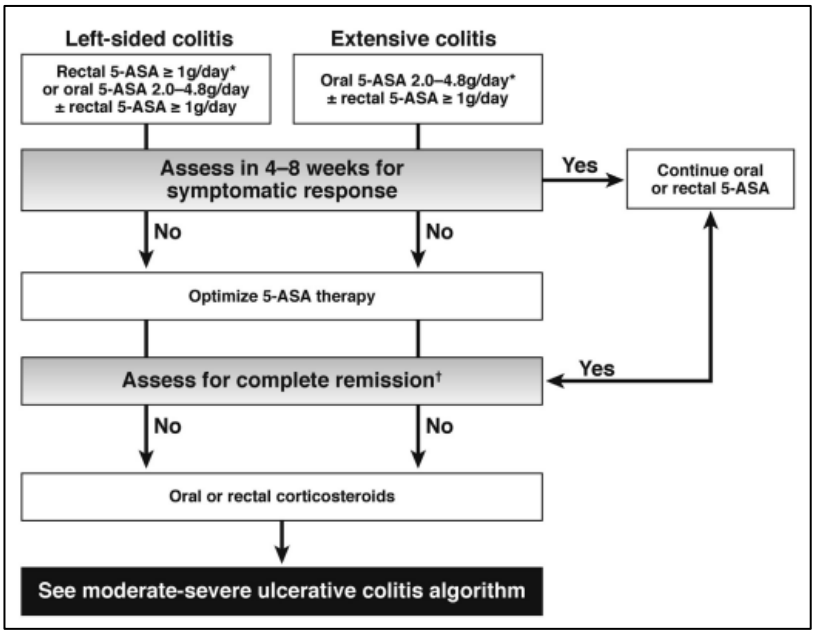


Figure 3: Consensus-guided algorithm from Toronto Consensus Practical Guidelines for the management of mild-to-moderate UC (82). †Between 4-12 months after initiation of treatment.

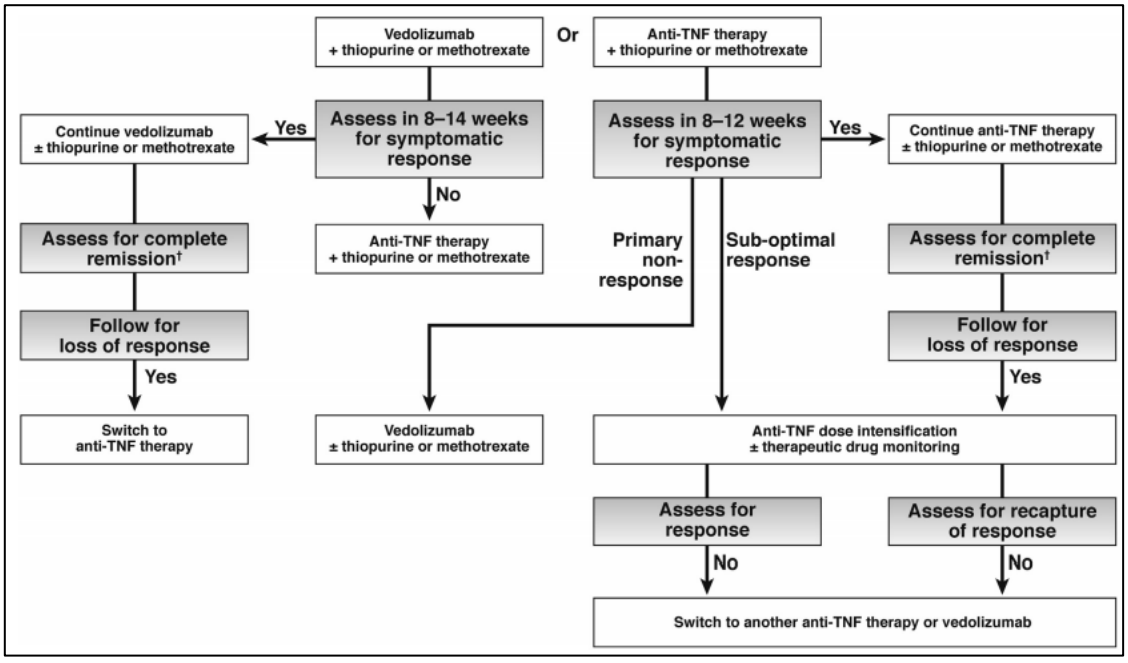


Figure 4: Consensus-guided algorithm from Toronto Consensus Practical Guidelines for the management of moderate-to-severe UC (82). *Not appropriate for patients who fail to respond to 5-ASA. † The role of dose intensification and therapeutic drug monitoring with vedolizumab therapy is uncertain. ‡ Between 4-12 months after initiation of treatment.

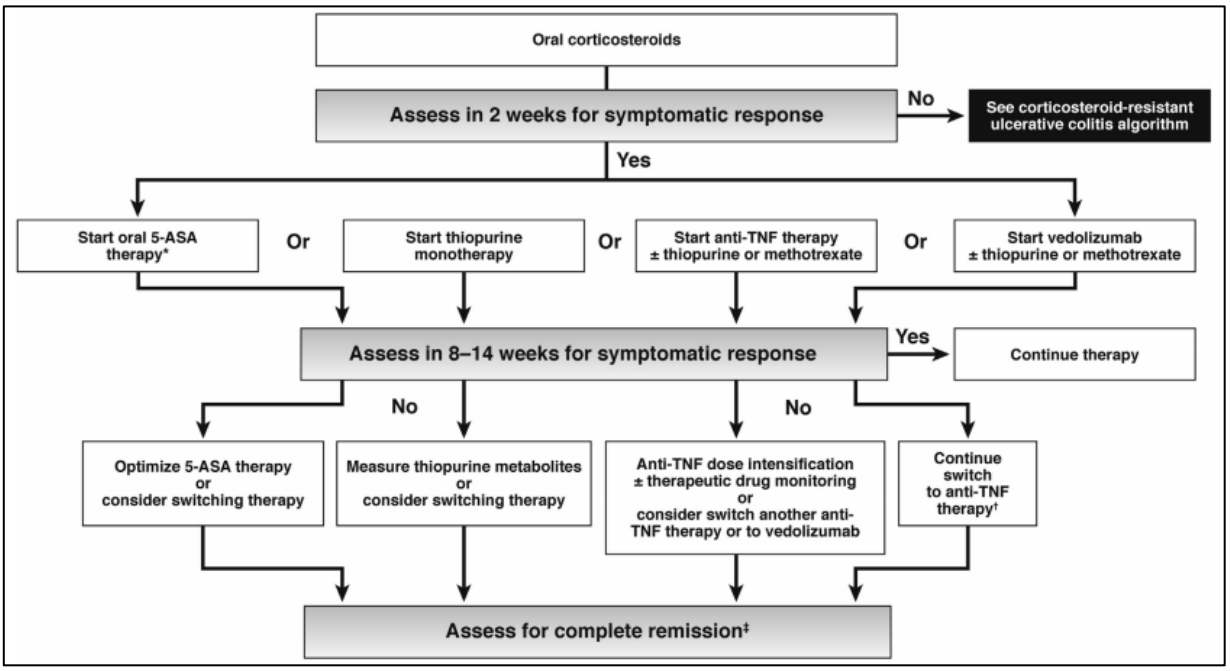


Figure 5: Consensus-guided algorithm from Toronto Consensus Practical Guidelines for the management of corticosteroid-resistant/dependant UC (82). 5-ASA is not appropriate in these patients. † Between 4-12 months after initiation of treatment. When complete remission is achieved, therapy should be continued.

These are the different steps in the clinical management of UC available nowadays. They form the classical therapeutic pyramid of UC, which it has the surgery intervention at its peak. Nevertheless, as the price of biologic treatments is becoming to decrease, the shape of this pyramid is changing and, in some centres and guidelines, the immunosuppressive therapy with thiopurines is being replaced for the biologics as a second step drug when the 5-ASA therapy fails. However, one must consider that after the failure of an anti-TNF agent a generally reduction in response to each successive drug occurs and, sometimes, the best option is the surgery. (58).

Crohn's disease management

First step: induction of remission treatment. The management of the active disease is going to be influenced by the localization of the disease.

- Ileocecal CD (L3):

- Mild-moderate activity: oral budesonide, 9mg daily. It is inferior to conventional steroids, but it has fewer side effects. Mesalazine has not shown a significant benefit over placebo. Systematically acting antibiotics must be avoided and the only one that can be considered in selected patients is rifaximin, a non-absorbable antibiotic well-tolerated (55).
  If no response is achieved with this strategy the second line must be systemic corticosteroids or an early introduction anti-TNF strategy in case of patients who have shown steroid-refractory in the past, in order to minimize the use of systemic steroid drugs (55).

- Sever activity: the first line drugs are systemic corticosteroids (oral prednisolone or intravenous hydrocortisone). If poor prognosis is assessed (by defined clinical-criteria), or the patient have antecedents of steroid-resistance, anti-TNF should be introduced as an earlier therapy line (55). If the patient does not respond vedolizumab/natalizumab, followed by USK, must be considered (83).
- Isolated involvement of the rectum or sigmoid colon can be treated with topical drugs or 5-ASA or budesonide enema formulation (25).

- Colonic CD (L2): the recommended treatment for active colonic CD are intravenous systemic corticosteroids or anti-TNF directly if there is a history of relapse (55).

- Extensive small bowel disease (+L4): the initial treatment are intravenous systemic corticosteroids versus anti-TNF if there are features suggesting poor prognosis (55).

In any case, the treatment of an active CD should include bowel rest and fluid resuscitation.

Second step: maintenance of remission treatment.

There are two different strategies when facing CD: "step-up" therapy and "top-down" therapy. The first one refers to the use of low-potent drugs, such as 5-ASA, with fewer side effects and progress to more powerful ones according to clinical response. The second one, prefers to start with potent medication, such as thiopurines or biologics, in order to achieve remission. The choice of one or other depends on the severity, localization of the disease and the history of the patient. There are three major factors to take in account:

- The course of the disease: initial presentation and frequency and severity of the flares.
- Extent of the disease.
- Previous experience from other treatments for CD, in case of relapse: effectiveness and tolerance.

Therefore, the following recommendations have been done following the statements above and approved in the *"3rd European Evidence-based on the diagnosis and management of Crohn's disease of 2016"*(55):

- Maintenance treatment after a first presentation of CD (with remission achieved using corticosteroids): the recommended drugs are thiopurines (azathioprine) and methotrexate. In some cases, an observational strategy (no-pharmacological treatment) can be consider.
- Maintenance treatment after relapse: "step up" strategy. Treatment escalation with the next available drug (thiopurines/MTX → anti-TNF → switch if fail → swap if fail: vedolizumab → swap: ustekinumab). Surgery should be avoided because of the high taxes of recurrence associated.

Before changing the drug used, the physician must always evaluate the adherence to the therapy, as well as the presence of objective signs of active inflammation.
Moreover, in order to confirm the loss of response to anti-TNF agents, the first step needed is a dose optimisation of the drug followed by anti-TNF levels and anti-TNF Ab assessment by blood test.

- Maintenance treatment in aggressive/severe CD: "top down" strategy.

The physician cannot forget the importance of enrolling the patient to stop the smoking habit, which is one of the factors related to the flare ups of CD (55).

There are no evidence of the use of pre or probiotic in CD (55).

Finally, regarding antibiotics in CD is worth mentioning when use them. The strongest recommendation is for the treatment of associated perianal disease (ciprofloxacin). There is weak evidence related to the use of rifaximin, which seems to be effective for the treatment of mild-to-moderately active CD (55).

Surgery

The evidence regarding surgery in IBD comes from case-series reports and there is an important lack of controlled trials about this topic. Furthermore, the response ratio to surgery is considerably different in CD compared to UC.

Surgery in CD: CD tends to reappear to the surgical anastomosis after some time. For that reason, surgical strategy is the latest to be applied in these patients. Approximately, 40% of them undergo through it at 5 years of the initial diagnosis, whether this percentage increases to 70% in the 10 years' time. For that reason, before the surgery, other interventional techniques are recommended, mainly endoscopic dilatations in symptomatic strictures. Nevertheless, it is truth that the effective ratio of them is low (55).

Surgery in UC: it is estimated 35% of patients with UC will receive a surgical intervention. The response ratio is much better than the seen in CD. The surgery technique consists in a two times intervention: 1- subtotal colectomy with transient ileostomy; 2- proctectomy and pouch creation.

Microbiota targeting treatments

Two therapeutic strategies are worth mentioning before closing this topic: probiotics and faecal transplantation in IBD.

Probiotics: the use of living microorganisms which exerts a beneficial effect in the host in order to modulate their intestinal microbiota has been examined for a long time now. It seems to be more effective than placebo in the remission maintenance of UC patients, some studies even claim their effectivity as an induction treatment (35). Nevertheless, there is no strong evidence regarding CD, and it seems probiotics have no advantage

over placebo in this disease. Therefore, more and well-designed studies need to be done (84).

Faecal transplantation: it is one of the newest therapeutic options proposed for IBD. It seems to have potential benefits for these patients, nevertheless, the difficulties in the donor selection (microbial, genetic and immunologic factors) and the bias in the studies made until the moment make the results difficult to interpret and, therefore, not allow the application of this therapy in the clinical practice yet (35).

Relapse assessment and management

As mention in previous paragraphs, IBD is a chronic disease with a relapsing-remitting course. The main goal of the therapeutic measures used in the management of these patients is to induce and maintain the remission of the disease, which is, to avoid its progression and the chronical inflammation which goes with it.

Nevertheless, despite an effective medical therapy, with the consequent effects of it, it is possible a degree of subclinical inflammation persists within the mucosa of the gut, which can progressively increase until reaching a critical intensity when the flare-up will appear (relapse of the disease).

Moreover, there are some clinical and environmental factors that increase the risk of relapse, such as: IBD localization (perianal disease, extensive colitis…), young age at diagnosis, female sex, some drugs (NSAIDs, oral contraceptive pills…) and a short period of remission before relapse, among others (85).

Once said that, how physicians can predict the relapse risk of this patients?

There are different tools used in order to achieve that goal. The general algorithm used when facing the suspicion of an IBD flare-up is reflected in the (Figure 6).

Ones of the most used tools are probably the clinical predictor scores: CDAI for CD and UCAI for UC (Annex 5).

Apart from that, there are different laboratory parameters that have been assessed for the IBD relapse prediction. The list is large, so only the main ones used in the clinical practice are going to be explained.

The first parameters assessed, for its simplicity and experience in the clinical practice, are CRP and ESR: both are serum inflammatory biomarkers that reflects the inflammatory activity over the organism. Values above 20 and 15 respectively, have been proved to be associated with 8-fold increase of relapse in some series (86). Nevertheless, its effectivity as predictor biomarkers have been proved for CD, and it remains unclear their role in UC.

Patients with IBD in remission can suffer from a relapse at any time due to different causes. One of the biggest problems is the timing of this fact is unpredictable. In this scenario is where faecal calprotectin is of most useful. Its role is to detect the initial

inflammatory response upregulation in the window period in between the remission state and the appearance of symptoms due to relapse of the disease (51). It is true the gold-standard test in order to assess relapse of the disease is the endoscopy examination and evaluation of mucosal healing. However, it represents a more invasive, expensive and labour-intensive test than faecal calprotectin, which is non-invasive and low-cost. Moreover, the use of faecal biomarkers instead of FCS, in order to assess active inflammation, avoid the risks associated with the procedure, such as infections and intestinal perforation (51). As mentioned before, faecal calprotectin has proved to have a high NPV to assess IBD. Therefore, when an IBD flare-up is suspected calprotectin must be included in the initial study of the patient. If the results are negative, IBD relapse diagnosis is discharged and other ones should be considered. If positive, it increases the odds of a relapse, but more studies are needed to be done.

One of the biggest problems of the clinical indexes and laboratory parameters in the monitorization of quiescent IBD patients is its incapacity to recognize the patients before the inflammation is established. This fact could seem obvious but is important to consider because there is no current efficient biomarker used to anticipate the establishment of inflammation. And once this happens, the symptoms are usually present as well as the gut damage (51). Furthermore, none of the mentioned tools used in the relapse prediction of IBD have showed its usefulness alone, which mean they need to be assessed together.

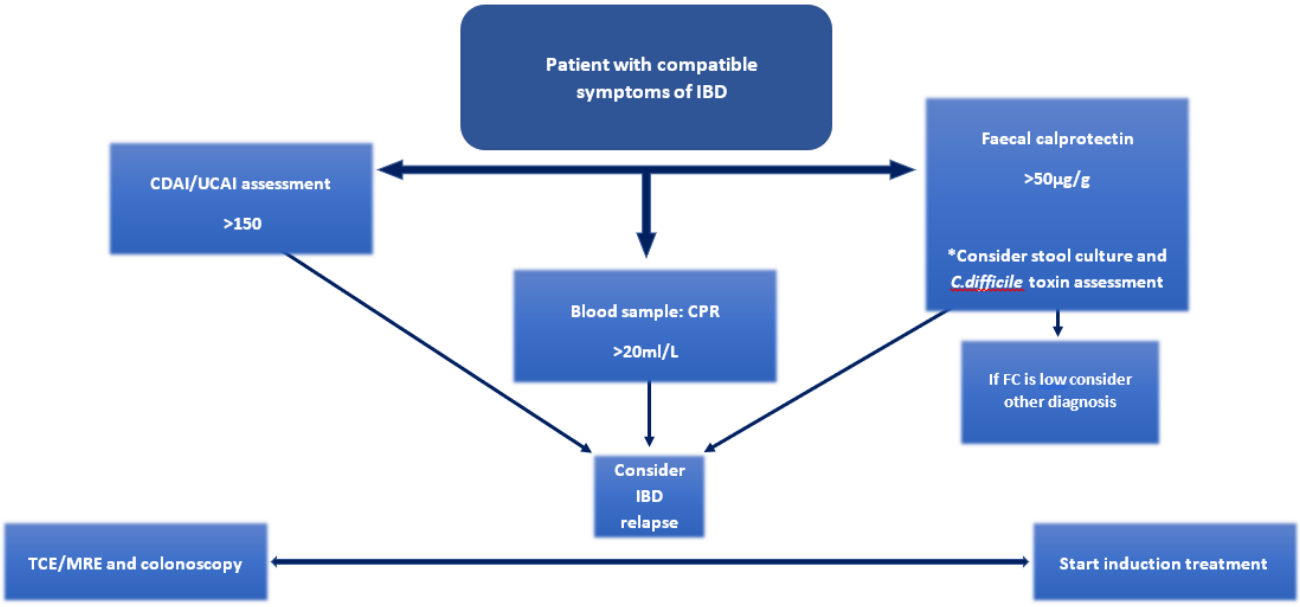


Figure 6: diagnosis assessment of IBD-relapse suspicion.

New biomarkers studies for assessing relapse: the role of microbiota assessing

A new target for the assessment of IBD relapse is the alteration on gut microbiota composition. The disbalance in specific species of bacteria, such as *Clostridiales*, have

shown to be able to predict the response to infliximab treatment and anticipate the time of relapse of the disease (31,32).

Moreover, the bases behind the test used in this study are the same. The evidence related to gut microbiota disturbances, as well as different microbiological footprints based on the disease stage (activity vs remission), allows to create a test capable of assessing the relapse risk of IBD.

3.1.2 IRRITABLE BOWEL SYNDROME

Concept and epidemiology

Irritable bowel syndrome is a functional disorder of the GI tract. It is defined as the presence of abdominal discomfort or pain and alteration of bowel movement habits. It represents the most prevalent functional disorder of GI tract.

The prevalence of IBS in the general population is high and can vary between 5 to 20% (median of 11'2%), with different geographical distribution (87,88). Since the introduction of Rome IV diagnostic criteria, the prevalence for IBS was thought to be between 10-15%, nevertheless, recent studies using the new Rome IV criteria point to a lower prevalence, around 5-6% (32). This fact could be translated in different ways. On one hand, the impact for the people suffering from IBS who see their quality of life frankly deteriorated, as well as their social and professional life, as IBS represents one of the main causes for absenteeism from work (3). On the other hand, the social and economic repercussion of the syndrome and the huge price that represents for the sanitary system.

Most cases are diagnosed before 45 years old, so the high incidence of IBS is found in young adults, particularly in women, who suffer from this disorder around 2 or 3 more times than men.

Nevertheless, when we fix our attention over IBS-symptoms in patients with quiescent IBD, the prevalence of the entity is even higher, 35% to 45% according to some articles (87).

Aetiopathogenesis

The aetiology and the pathogenesis of IBS are not well understood. The role of genetics is not clear, whereas it seems to play a role in the disease, there is little information about it. The main alterations found until today are polymorphisms of genes such as *cadherin 1, cell division cycle 42, IL6* and *10* genes among others (89). However, there are other known factors related to the disease and there are thought to have a major role in the development of IBS:

- Epithelial barrier alteration.
- Food antigens.
- Bile acids.
- Gut microbiota.

The interaction between them and with the organism may produce an abnormal response and a dysregulation in the sensorimotor functions through an aberrant malfunction of the enteric nervous system, the brain-enteric axis and the gut immune system.

The first in the list, epithelial barrier alteration, it refers to morphological and physiological changes in intestinal permeability due to a defect in the tight junction proteins of the enterocyte. This fact creates a "leaky" gut barrier which allows the infiltration of low-grade immune cells infiltration of the gut mucosa in response to the increase exposition to bacterial antigens and food substances. The cause underlying the alteration of junction proteins is not clear. It has been suggested the role of bacterial-mediated immune response that leads to a condition of chronic inflammation, as well as proteasome-mediated mechanism (90–92).

As for bile acids, some patients have been found with increased levels of faecal bile acids, causing an acceleration of colonic transit and the induction of diarrhoea and visceral hypersensitivity. The cause seems to be an increase excretion of a surrogate for bile acid synthesis, with genetic implications (93–95).

The role of gut microbiota in different intestinal disease has gained an especial interest in these current years. One of them is IBS, in which gut microbiota seems to be disbalanced compared to healthy individuals. The fact of having a different bacteriological, as well as viral and fungal, population allows to try to get a specific microbiological signature of the disease. Thanks to the assessment of thousands of stools samples in different studies, the microbiota profile for IBS is starting to be more evident. Compared to the general and healthy population, the whole microbiota ecosystem is less rich, and it seems to be an increased number of *Parabacteroides distasonis, Bacteroidetes* and *Verrucomicrobia* spp. and a reduction of *Lactococcus, Firmicutes, Actinobacteria* and *Pseudomonas* ones. Furthermore, it seems to be two defining species in IBS, which are *Oscillospira* and *Erysipelotrichi* (32).

Moreover, not only phylogenetic studies have been done, animal model studies have shown the induction of IBS-like symptoms and visceral hypersensitivity through the colonization of germ-free animals with microbiota from IBS patients (96).

One can oppose to the role of microbiota in IBS claiming it is an unstable and variable parameter modified by diet and drugs. Nevertheless, the microbiotic signature of every individual is quite stable and specific dietary interventions aiming the modification of the microbiota per se are needed in order to interfere in the balance of it (97).

Apart from the disbalance in the populations *per se*, the importance of gut microbiota is also in the metabolites pathways in which some species are involved. Particularly, the

two most relevant ones are SCFA production, due to the fermentation of non-digestible foods, and protein degradation. SCFA are needed for the correct function of the gut cells. They served as energetic fuel for enterocytes and contribute to the modulation of host immune response, thanks to their ability to induce the production of antimicrobial peptides and to modulate the number and functions of regulatory T cells. However, not all SCFAs are desirable, and if a dysregulation on its production occurs that could lead to an alteration of the gut immune system, the stimulation of T cell lymphocyte and the disbalanced between pro-inflammatory and anti-inflammatory mechanisms, producing a low-grade intestinal chronic inflammation which can lead to changes in intestinal tissue (98–101). In the case of IBS, it seems that SCFA stool concentration is increased compared to healthy population, a fact that supports the dietary intervention as a therapeutic strategy in IBS (see below) (102). On the other hand, a high protein diet can lead to the production of excessive toxic substances that the microbial population, particularly bifidobacteria, can cope with. These harmful metabolic products stimulated the immune system and lead to a state of inflammation (103,104).

The pathological pathways by which the mentioned factors produce IBS is not well understood either. Nevertheless, there have been proposed some ideas concerning that fact. Patients with IBS seem to have two main condition that predisposed and lead to the disease: an excessive increased motor activity, which can remind even 3h after the meals, and visceral hypersensitivity, mainly produce or associated with the food ingestion. The underlying cause by which the disfunction appears is not known but, in both, it seems to participate the serotonin pathways, a hormone which takes part in the regulation of intestinal motility and visceral perception, and it has been proposed that an increased production of 5-HT may contribute in the pathogenesis of IBS (105).

CNS regulation, behaviour and IBS

There is a notorious participation of the CNS in IBS. This affirmation is based on the high prevalence of anxiety and mood disorders in the patients suffering from the disease, as well as other chronic pain conditions in them. The evidence regarding the comorbidities related to IBS, as well as the exacerbation of the symptoms with high levels of stress and, in addition, the efficacy of CBT as a therapy for the disease, has led to assess the presence of a widespread dysregulation of the nervous system in these patients (105,106).

Thanks to functional neuroimaging techniques, it has been observed an excessive activation of the cingulum with the stimulation of the distal colonic region. This area of the brain is responsible for processes as attention and answer selection, which can justify the changes in pain perception. Moreover, the prefrontal cortex shows, as well, a preferential activation and can be related to the hyperalert state of the patients suffering from IBS and the increased visceral pain perception (105,107).

Even though the brain implication in IBS is known, more studies are required in order to assess the causality between them.

IBS and immune system

The role of immune system and low-grade chronic inflammation in IBS is thought due to the high prevalence of IBS-like symptoms in IBD patients in remission. Some studies show the increased presence of activated immune T cells and mast cells and its infiltration in the intestinal mucosa (108,109). The chronic inflammation may lead to an overexpression of TRPs, particularly TRPV1, in the enteric nervous system. These receptors are involved in the processes of hypersensitivity and hyperalgesia, both present in IBS (75).

Furthermore, IBS has also been related to gastroenteritis-like infections. It has been described the debut of IBS in patients after going through an intestinal infectious process, called post-infectious IBS. The main risk factors related with this form of the disease are gender, in this case women, particularly less than 60 years, long infectious process, toxic bacteriological strain and mood disorders. The main pathogen associated with post-infectious IBS is *Campylobacter*, which can produce long-term changes in intestinal epithelium and end in the disease (105)

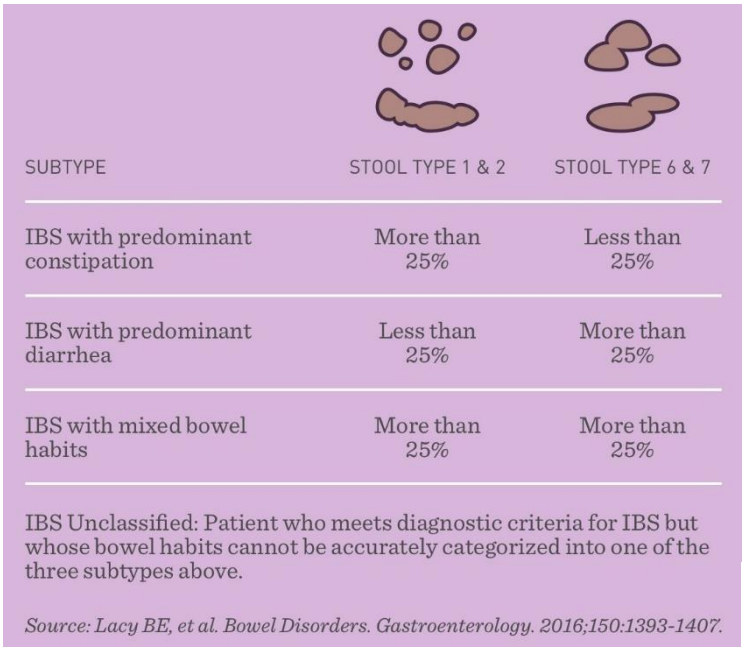
Signs and symptoms

The lead symptoms are abdominal discomfort or pain and alteration of bowel movement habits, in form whether of diarrhoea or constipation.

Abdominal discomfort or pain is the main symptom for IBS, and it is highly related to bowel movement, it lessens with the defecation or it presents related with stool irregularities and bloating. Its characteristics varies among the patients but is typically described as colic episodical pain. It does not usually relate with malnutrition or sleep deficiency, because the pain develops during the wakefulness period.

The other defining symptom is stool alterations. According to the predominant irregularity four different subtypes of IBS have been defined (Figure 7): predominant constipation (IBS-C), predominant diarrhoea (IBS-D), mixed (IBS-M) and unsubtype (IBS-U). Even though the manifestation of the disease is different, the management of it does not differ and, even more, the different subtypes overlap frequently (110). The presence of blood is not a characteristic feature of the diarrhoea related with IBS, except in the presence of haemorrhoids. Nevertheless, the stool can have mucus in it, sometimes in large quantities.

The symptoms have an intermittent behaviour, and the syndrome goes through periods of remission and relapse. It is frequent to find other somatic and functional disorders related to IBS, such as fibromyalgia, back pain, headache, chronic pelvic pain syndrome, and overactive bladder (111,112), as well as, other gastrointestinal disorder, dyspepsia, pyrosis and gastroesophageal reflux, incontinence and, as it is the purpose of this study, quiescent IBD, among others (113). Moreover, there are a high prevalence of psychiatric comorbidities in IBS patients compared with the general population (114).



SUBTYPE	STOOL TYPE 1 & 2	STOOL TYPE 6 & 7
IBS with predominant constipation	More than 25%	Less than 25%
IBS with predominant diarrhea	Less than 25%	More than 25%
IBS with mixed bowel habits	More than 25%	More than 25%

IBS Unclassified: Patient who meets diagnostic criteria for IBS but whose bowel habits cannot be accurately categorized into one of the three subtypes above.

Source: Lacy BE, et al. Bowel Disorders. Gastroenterology. 2016;150:1393-1407.

Figure 7: IBS subtypes

The intensity of the clinical manifestations varies within the person from a wide range. Nevertheless, IBS can cause an important reduction in the quality of life of the patients and became a very limitational problem.

Diagnosis

Until today, there are no diagnostic markers validated for a positive diagnosis of IBS. Therefore, the entity is defined by clinical criteria, called Roma criteria. Nowadays, these items have been revised in four occasions, and IBS is diagnosis using the Rome IV criteria, uploaded in 2016 (Annex …). All the different Rome criteria have in common the presence of abdominal pain/discomfort associated with bowel habit alterations (115), although, Rome IV criteria introduce new features in its statements. Firstly, the term discomfort has been removed because its different meanings according to the language used and the difficulty to make an accurate distinction between it and pain. The next change is in the abdominal pain frequency required. It has been increased from 3 days per month to one day per week on average. In third place, bloating and distension have been recognised as common symptoms of IBS. And lastly, the new criteria state, explicitly, the different IBS subtypes, based on predominant bowel habits (116).

Even though the diagnosis of IBS relies on fulfilling clinical criteria, it also requires ruling out organic causes which can produce similar symptoms. Therefore, once the patient meets the Rome IV criteria, additional test must be performed leaded by the prevalence of the symptoms and the clinical history of the patient (117).

The evaluation of any patient suspected from IBS should include:

- Detailed anamnesis: not only to verify the patients meets Rome criteria, but also to find out other symptoms which help the diagnosis, such as postprandial worsening/exacerbation of the symptoms. Moreover, a detail clinical history recording other gastrointestinal symptoms, as well as other non-gastrointestinal manifestations, must be performed (111).
- Physical examinations: particularly abdominal examination, in order to demonstrate the absence of objective findings, a fact that supports the diagnosis of IBS. One cannot forget the examination of the whole body and the digital rectal examination and perianal inspection, to rule out any masses, hide blood or perianal fistulas (118).
- Laboratory tests: the assessed parameters must complement the clinical suspicion. Two main items are mandatory to differentiate IBS from IBS: CRP and faecal calprotectin. If both are low (less than 0'5mg and 40µg, respectively) the diagnosis of IBD is discarded (119). Other helpful data that can be included in the analytical exam of the patient, according to the symptoms, are coeliac profile, thyroid hormones (in case of IBS-D) (120). If there is a doubt about the presence of a gastrointestinal infection stool analyses should be performed.

If these three steps are suggestive of IBS, and there are no alterations in the laboratory tests, no more tests are recommended, so there is no need for invasive procedures (121).

However, there are sometimes where the clinician can find the so-called alarm features, which is symptoms or laboratory abnormalities that may suggest organicity. In this scenario, other tests are required, and the selected one is a colonoscopy. Even though, it is important to make clear the alarm features have not showed to improve the performance of IBS diagnostic criteria, so they are used in order to select the patients who need further diagnostic testing. Among the ones chosen, a substantial proportion will not have an underlying condition of gastrointestinal tract (122,123). That fact shows the need to use other criteria and techniques in order to not perform unnecessary and invasive tests to patients without a clear indication, as in this case.

The use of complementary tests depends on different factors of the persons, being age and severity of symptoms two defining items in order to proceed with further investigations on the patient. As mentioned above, colonoscopy, particularly rectosigmoidoscopy, is a mandatory test when organicity is suspected, nevertheless, it is not the only one. When IBS presents predominantly with diarrhoea (IBS-D) a stool analysis is required, in order to rule out a parasitical infection.

Treatment

The management of IBS is difficult. There are two main features that make the treatment challenging. The first one is the still unknown IBS ethiology and pathogeny, which limits the useful drugs for it. The second one is the psychological burden associated with this disorder. An important percentage of patients (50-70%) suffer from

other somatic and/or psychological symptoms apart from IBS. Moreover, only around the half of patients with IBS seek for help, so there are a big part of these patients living and dealing with the syndrome without treatment (124–126).

So, once said that, how one can deal with IBS (127)?

There is no magic formula, but the optimal management of the disorder goes through different layers of strategies.

- Effective and strong patient-provider relationship.
- Education and reassurance about the management and the course of the disorder.
- Dietary interventions, which is nutrition management.
- Behavioural and psychological therapies, which need to include aspects of cognitive and interpersonal therapy.
- Pharmacotherapy: antispasmodics for abdominal pain, antidiarrheals (IBS-D) and laxatives (IBS-C), as well as in some cases SSRIs can be effective.

Nutritional intervention

The exacerbation of symptoms after food ingestion is one of the most frequently reported inductor factors. Moreover, postprandial symptoms and the fear of their occurrence, which is anticipation anxiety, are two problems which contribute highly in the reduction of the quality of life of these patients (128,129).

Although, there are no studies strong or reliable enough about the best diet for IBS patients. The role of gut microbiota in the disorder is becoming clearer as well as the alter reaction in front of some alimentary antigens.

The main role for nutritional intervention, with the information known until today, is to identify the main food that leads to exacerbation and help the patient to plan a balanced diet free from them (130). One of the main targets in IBS is FODMAPs, which is, fermentable oligosaccharides, disaccharides, monosaccharides and polyols. A low FODMAP diet has been suggested as a therapeutic tool for IBS, but more studies and strong evidence is needed. The theory beneath this strategy is to stop the ingestion of food which represents the main supply for some of the bacteria though to be involved in IBS pathogeny. As always, an efficient treatment based on modifying dietary habits without the need of taking any drug is desired, but the low FODMAP diet is difficult to fulfil. It requires the elimination of garlic, onion, wheat, lactose, fructose, wine and beer, among others (131,132).

A part from diet modifying strategies, some studies have found efficacy in the use of probiotics in IBS, but more research is need to be done because there are no supportive data enough regarding their use or the prescription of prebiotics as well (133–136).

Pharmacotherapy

There are two different strategies when talking about drugs used in IBS. The first one, and the most widely used, are the ones which aim for the symptomatology related with IBS, bowel habits alteration and/or abdominal pain. The second one is newer, and the target is the gut microbiota. This therapy is based on studies that has shown a different microbiome proportion in healthy individuals compared to persons with IBS (32).

Symptomatic treatment:

- Antispasmodic drugs: anticholinergic drugs have shown to be effective to temporally control abdominal pain (painful colic related with intestinal spasms). These drugs are more effective if used before the manifestation of the pain, so they must be taken 30min before the meals. They are not exempt of side effects and one must be careful if they are used in elderly population (127,137).
  Another antispasmodic with demonstrated effectivity in reducing IBS symptoms is peppermint oil, which main function is, as the anticholinergic, to inhibit smooth muscle contraction (138).
- Low-dose antidepressants: these drugs do not have the indication per se for IBS, but they have been used in patients with chronical pain who do not respond to antispasmodic and dietetical intervention, which is, their use is off-label. SSRIs are the anti-depressants of choice in IBS-C, because of its low side effects compared with other drugs of the same category (such as TCAs) and the capacity of accelerating the intestinal motility, whereas TCAs are used in IBS-D, particularly imipramine, because of their evidence and ability to inhibit or diminish intestinal rhythm (127).
- Laxatives and motility accelerants: used in IBS-C. The first line drugs are simple laxatives and, if the strategy fails, one must move forward to second line ones such as linaclotide or 5-HT4 (it has been alarms related to its cardiovascular toxicity and some drugs has been removed from the market) (139–141). Lactulose must be avoided due to its bas tolerance and worsening of symptoms (bloating and discomfort) (127).
- Antidiarrheals: used in IBS-D. The chosen drug is loperamide. It is a μ-opioid receptor agonist with peripherical action, this is, does not cross the blood-brain barrier, so it has fewer side effects compared with other drugs from the same family (142). Another option is cholestyramine (Harrison).

<u>Psychotherapy</u>

The optimal treatments targeting biopsychological factors related with IBS has shown to diminish the rate of medical visits in the patients suffering from it. The association between the syndrome and anxiety and depression has been known for some years, and, even though there is not enough information about the pathophysiology beneath this fact yet, IBS is thought to have important psychological factors which influence in its appearance (143,144)

One of the main complains of people suffering from IBS is the feeling of incomprehension, whether from their physician and/or their relatives and friends, and

a positive and strong therapeutic physician-patient relationship helps to decrease IBS exacerbations (144).

The most studied model of therapy for IBS management, and the one with more evidence as well, has been cognitive behavioural therapy. Other options are hypnotherapy with gut-directed hypnosis, psychodynamic therapy and mind-fullness based therapy.

The guidelines recommend the start of psychotherapy if there is no response after 12 months of pharmacological treatment (127).

IBS and IBD: two different entities or two extremes of the same inflammatory process?

IBS and IBD are two well-defined entities which are different in terms of pathology, natural history, prognostic and therapeutic approach. Nevertheless, they share some similarities, particularly referring to the clinics and demographic distribution. Moreover, in both illness there are a relation with the alteration of gut microbiota, a dysbiosis in it. It is true, that the microbiological signature is not the same for both and, also, the decrease in the bacterial population is higher in IBD (145).

Even though the differences are understandable, the differentiation between the two of them become less clear when there are no signs of inflammation in IBD individuals, as in remission state, but there are still functional gastrointestinal symptoms.

Issues to consider in the potentially overlapping IBS-IBD:

There are several hypotheses regarding IBS and IBD, some of them defending its similar initial triggering and the shared clinical spectrum, and others opposing to the fact that the two illness are part of the same entity.

When comparing both, one must be aware of the similarities, as well as the differences between them.

The basic elements that support the relation between IBS and IBD:

- The higher prevalence of IBS-like symptoms in IBD individuals than in the general population.
- The clinical manifestations can be very similar, mainly when one compares IBS and IBD reactivation, in which abdominal pain is one of the most frequent symptoms in both types of patients (146).
- Similar familiar aggregation: IBD is more frequent in patients with relatives suffering from IBS (147). Moreover, some studies have investigated the existence of known IBD susceptibility loci in IBS. Regarding that, it seems that some gene polymorphisms, such as TNFSF15 gene, are more frequent observed in IBS patients than in the general population, suggesting a possible common predisposing mechanism in the two entities. However, more work and research needs to be done in this field (148,149).

- Shared predisposed factors, such as AGE, as well as the existence of gut microbiota dysbiosis, being more evident in IBD though.
- Stress and other psychological factors (such as depression) can act as flare-up factors in both entities. Furthermore, it seems, from patient report series, one third of patients with IBD respond to SSRI treatment, suggesting the implication of the brain-gastrointestinal axis as in IBS (150,151).
- The understanding of IBS as an inflammatory disorder, hence the possibility of a shared disease spectrum between IBS and IBD (two extremes of a similar pathogenic trigger). Signs of microinflammatory activity have been found in IBS patients.

Differences

- IBS does not seem to have a strong ethnic association as IBD. It has a worldwide distribution among rural and urban areas comparing with IBD, which is more prevalent in West countries and urban areas.
  IBS is more prevalent in women than men, the opposite patron of IBD.
- They share the fact that bacterial infections could lead to the initiation or relapse of the illness.
- Calprotectin levels are normal in IBS patients, whereas are elevated in IBD patients with inflammatory activity (>100µg/g; high specificity, 100%, sensitivity of 71%, high PPV, 100%, and lowe NPV) and lows in the patients in remission (<50µg/g; high sensitivity, low specificity, as well as low PPV and high NPV)(145). There is a grey zone when referring to calprotectin levels. Values between 50-100µg/g are not evident, and can reflect no-inflammation, subclinical inflammation or inflammation per se.
- The therapeutic management is extremely different. Whether IBS treatment is based in symptomatic relieved, diet and trigger control (dietary strategies, SCFA therapies, antidepressants and mindfulness therapy), IBD management involves a hierarchical approach based on top-down/bottom-up strategies with potent drugs and strict controls of its efficacy.
  Drugs used in IBD have not been properly studied in IBS management.

Prevalence of IBS-like symptoms in quiescent IBD patients

There are few studies focused on the real prevalence of IBS in IBD patients. Most of them refer to the incidence of IBS-like symptoms in IBD patients, without finishing the calculation of how many of them turn out to have a real IBS concomitant to its disease. Moreover, the studies which do try to stablish this fact have heterogenous results, and the prevalence differs from 20% (152) to 39% (146). CD patients in remission have a highest risk than UC ones of having IBS-like symptoms, particularly among women (108). It is not clear whether the high prevalence of IBS-like symptoms could be explained by casual overlap alone, or may be the result of clinically undetected, low-grade inflammation versus visceral hypersensitivity and mast cell infiltration induced by IBD (108).

3.1.3 GUT MICROBIOTA

The microbes inhabiting the human body, particularly those developing in the human gut, are being pointed out as important players in multiples aspects of human physiology and in the pathogenesis of several diseases, such as obesity, metabolic syndrome, inflammatory diseases and cancers, among others. The main pathologic pathway in which the microbiota takes part in it is still unclear, but it seems to be related with a breakdown in the homeostasis between microbiome and immune system, which leads to an increased risk of immune-mediated disease development against the host microbiota (153).

The gastrointestinal tract harbours 100 trillion microbial cells approximately, including bacteria, viruses, fungi and protozoa, more than the owner cells themselves. They are grouped by species or phylotypes and constituted the intestinal or gut microbiota, their collective genome known as microbiome, which includes more than 1000 species of bacteria (154). Among them, around 99% of intestinal bacteria belongs to four main phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* (155).

Even though there is no consensus on when it is placed the start point of the microbiota establishment in the gut, whether it is *in utero* or, more accepted one, it begins just after birth, it is clear its evolution through the person development. Microbiota composition in the new-born varies according different factors, some of them being delivery mode, feeding pattern (breastfeeding versus formula), hygiene and sanitary living conditions and use of drugs (especially antibiotics). During the first three years of life human microbiota composition fluctuates and changes among different colonization states until it reaches a proportion that resembles the adult one, which is mainly constituted by bacteria, particularly *Bacteroidetes* and *Firmicutes*, being the most prevalent phyla (154,156).

Although human microbiome, and especially gut microbiome, change during life influenced by factors such diet, lifestyle and environment, it is considered relatively stable ones the individual reaches the adultness (157). Even though it is true that there are permanent fluctuations in gut microbiota (diet, drugs, changes in physical environment…), it tends to return to its original compositional pattern (resilience) (158).

Microbiota functions

Gut microbiota develops multiple functions within the organism. The relationship between the two entities has existed for millennia and the microorganisms conforming the microbiota has coevolved with human hosts. Therefore, they have formed a wide variety of symbiotic interactions, some of them not yet understood or even known, which contribute to maintain human health and homeostasis. An alteration of gut

microbiota composition and, consequently, in its function is known as dysbiosis and it is thought to be one of the pathogenic pathways involved with some diseases such as IBD and IBS (35).

The role of gut microbiota in the organisms can be grouped in 3 main categories (159):

- Nutrition: vitamin synthesis (K and B) and SCFAs production (acetate, propionate and butyrate).
- Immune development: development of the immune system, IgA production, modulation of T cells and regulation of T-helper cells balance (Th1/Th2, Th17) and reinforcement on the barrier function.
- Host defence: colonization resistance (through different pathways, nutrition competition, for example) and production of anti-microbial factors (bactriocins, lactic acid…).

Nutrition role

Two main functions related to nutrition role of the gut microbiota:

- Vitamin synthesis: vitamin K and water-soluble B vitamins, specially from *Bifidobacterium* species (160).
- SCFA production: one of the main energy sources for colonic epithelial cells. Bacteria, such as the ones belonging to *Firmicutes* and *Bacteroidetes* phyla, produce SCFAs from indigestible o resistant starch carbohydrates, commonly known as dietary fibre. Three main acids are produced: butyrate, which is a primary energy source for colonic cells, and acetate and propionate, both are transported to the blood and become available in the systemic circulation (161).

Immune role

The relationship between gut microbiota and immune system is complex and, at some points, not-well understood.

Nevertheless, it is known the reciprocal relationship between them, which is, gut microbiota contributes in the maturation of host's immune cells and, in turn, immune system shapes the structures and function of gut microbiota, selecting some species over others.

A lot of evidence regarding this fact comes from studies with free-germ animals, mainly mice, which have shown a decreasing in the number of intestinal lymphocytes, lack of maturation of lymphoid tissues and diminished levels of IgA and some antimicrobial peptides (162–164).

Other immune related functions of gut microbiota are the modulation of T cells, especially regulatory T cells (T-CD4+) and the regulation of T-helper profile, mainly through the development of Th17 cells. Some bacteria, mainly the ones producing butyrate acid, interact with the immune system and induce the differentiation and expansion of Treg. cells, which allows to supress the excessive response of the immune

system towards the selected microbiome, in order to assure the production of butyrate. Different studies about this topic have been done, and one of the main species related with this function has been found to be *Faecalibacterium prausnitzii*, from *Clostridium* clusters. The importance behind this fact is the evidence of the decrease in the amount of this bacteria in patients with IBD, which could help to contribute to explain the pathology of the disease (165–168).

Host defence role

The third and final block of gut microbiota functions is the host defence regarding the intestinal tract. Free-germ animal studies have shown an increase of infections by intestinal pathogens in the ones without microbiota.

However, in which ways gut microbiota protect us against pathogens?

The main mechanism used in order to assure gut microbiologic homeostasis is called "colonization resistance". This term refers to the competition between different microorganisms for physical and nutritional niches in the gastrointestinal tract. By stablishing a stable population of commensal microbiota, the invasion of the intestinal tract by pathogens is prevented (169). There are two different mechanisms of action to enhance the mechanism just explained:

- Direct inhibition: nutritional competition (competitive exclusion) and synthesis of inhibitory substances (bacteriocin, for example a substance which targets spore-former microorganisms) (170,171).
- Indirect inhibition: activation of the immune response against pathogens. In this scenario, gut microbiota can be understood as an added part of the host immune system. It secretes substances that boost the immune system to produce substances against pathogens, such as antimicrobial peptides, IgA or REGIIIγ factor by Toll-like receptor pathways in the epithelial cells and dendritic cells ($TRL5^{+}CD103^{+}$) (172).

Gut microbiota in IBD and IBS

Inflammatory bowel diseases are characterized by abdominal pain and alteration of bowel habits, and there are thought to be linked to host genetics and gut microbiome unbalances (173,174). This alteration produces an aberrant interaction of the immune system of the host towards the abnormal gut microbiota in genetically predisposed subjects and under the influence of specific environmental factors.

IBS and IBD are both considered to have a thigh relation with a dysbiosis of gut microbiota, which is, an imbalance in the bacterial species leading to a loss of the intestinal homeostasis. Although they produce an inflammation as an alteration of the immune system response to microbiome, the result, as well as the alteration in the intestinal flora is different (32). There are different articles that have aimed to

understand the dysregulation of the gut microbiota in the inflammatory intestinal disease. From what is been published since today, it seems that the microbiological signature of the two diseases is not the same (Figure 8). Whereas IBD is characterized by a decrease in *Firmicutes, Bacteroidetes, Clostridium* (*F.prausnitzii*) and *Verrucomicrobia* species, IBS shows an increase in this two populations (even more than healthy individuals) and a reduction of *Actinobacteria* species (32). These changes seem to be less intense when comparing IBS individuals with subjects with IBD in remission, but there still were evident. The reduction in the abundance on gut microbiota it is produced at the expense of anti-inflammatory species and an increase in enterobacteria and other pathogenic microorganisms, such as sulphate-reducing bacteria, with the consequent increasing in the production of hydrogen-sulphate which contribute to the intestinal epithelial cell damage and chronic inflammation. The two most abundant species in healthy individual (*Bacteroidetes and Firmicutes*) appear reduced in IBD. Moreover, the number of some of the studied bacteria (mainly *F.prausnitzii*) has shown to be correlated with the risk of relapse after remission (35).

Gut microbiota and IBD

The microbiological signature for IBD is far from been understood, but an initial image of it is beginning to be clearer. The data obtained from faecal samples and biopsies, shows a reduction in the global diversity of gut microbiota, especially in *Bacteroidetes* phyla and *Verrucomicrobia* and an increase in *Actinobacteria*, compared to non-IBD individuals.

Dysbiosis and gut microbiota population seems to be different between IBD patients with active illness and patients in remission (inflammation vs “no-inflammation). The main population diminished in inflamed IBD are *Bacteroidetes, Firmicutes, Faecalibacterium prausnitzii,* Lachnospiracea and *Ruminococcaceae*, among others. Apart from the differences in the gut microbiota according to the disease activity, it seems to not exist a statistical significance cluster between UC and CD, even when stratifying IBD samples according to disease activity (32). To sum up, patients responding to IBD medical treatment has shown to improve their gut microbiota status, which resembles, at least more than IBD-active ones, to healthy individuals (32,35).

Gut microbiota in IBS

In the comparison between IBS and IBD inflamed mucosa, *Bacteroides, Lachnospiraceae, Parabacteroides, P. distasonis, Rikenellaceae, Coprococcus*, and *Ruminococcus* appeared increased in IBS, though *Enterobacteriaceae*, *Enterococcaceae* were reduced in IBS respect to IBD (32).

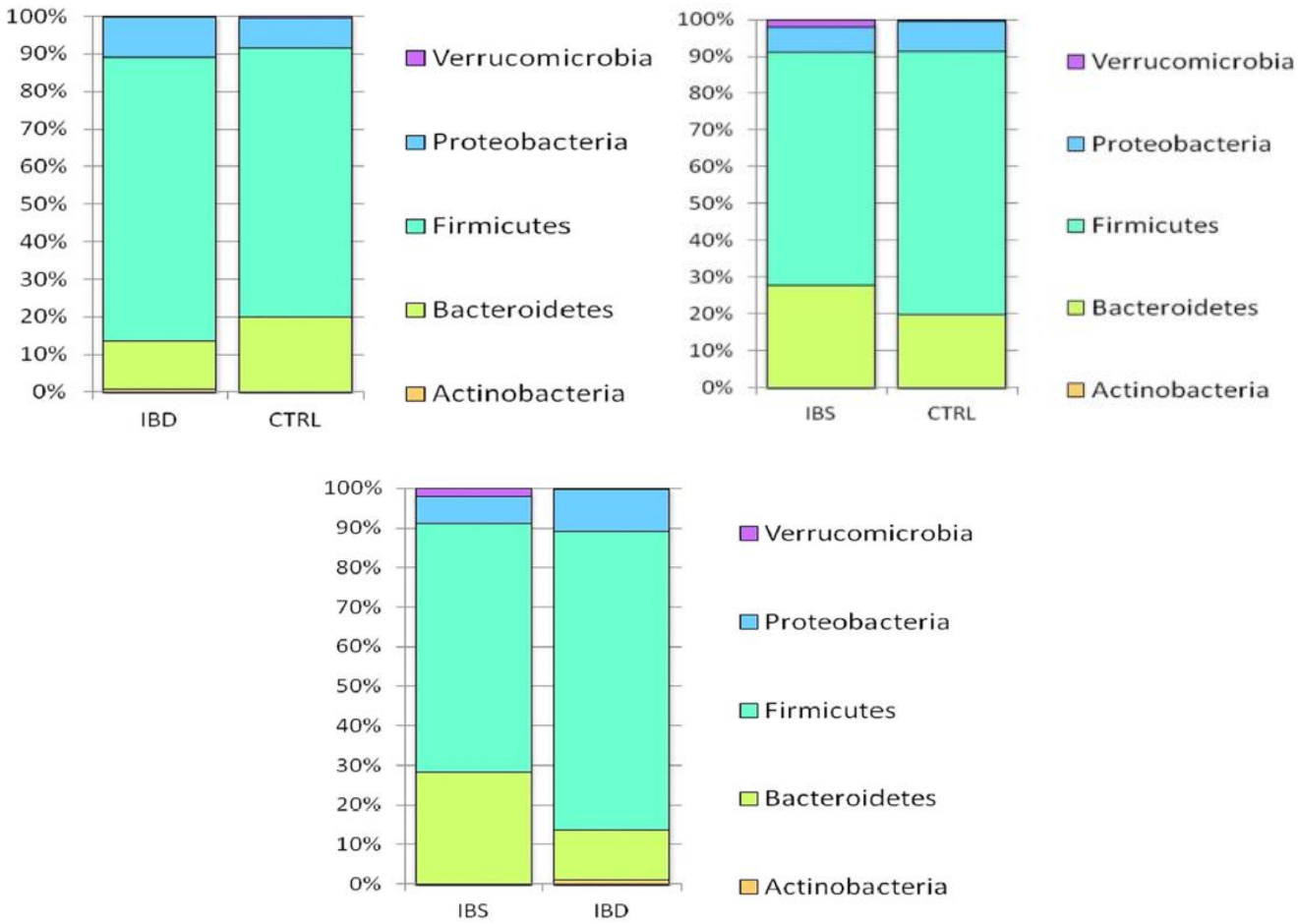


Figure 8: comparison between the differences in gut microbiota composition in healthy individuals (CTRL), IBD patients (IBD) and IBS patients (IBS), from stool sample analysis (32).

Gut microbiota and quiescent IBD

But what happens with IBD patients in remission? Some studies have shown patients who respond to the treatment, especially with anti-TNF drugs, will experience a progressive restoration of their microbiome especially in *Clostridiales* species. Therefore, patients who experience an IBD flare-up will show different gut microbiota profile compare with the ones who suffer from an overlapping IBS – IBD (31).

One fact that must be considered is the potential of thiopurine treatment to decrease gut microbiota diversity and change its composition as well (175). Therefore, patients with IBD in remission using this therapy could display a false result in the studied test.

A progressive model of gut microbiota alteration has been proposed in order to explain the evolution from an eubiotic state in a healthy individual to IBD microbiome profile, and some bacterial species characteristic from each entity are shown (*Erysipelotrichi* is a predominant specie in IBS patients and *Gammaproteobacteria*, *Enterococcus*, *Enterococcaceae* for IBD) (Figure9). Nevertheless, the information reflected in the image refers to the results obtained from biopsy samples, not from stool analysis (32).

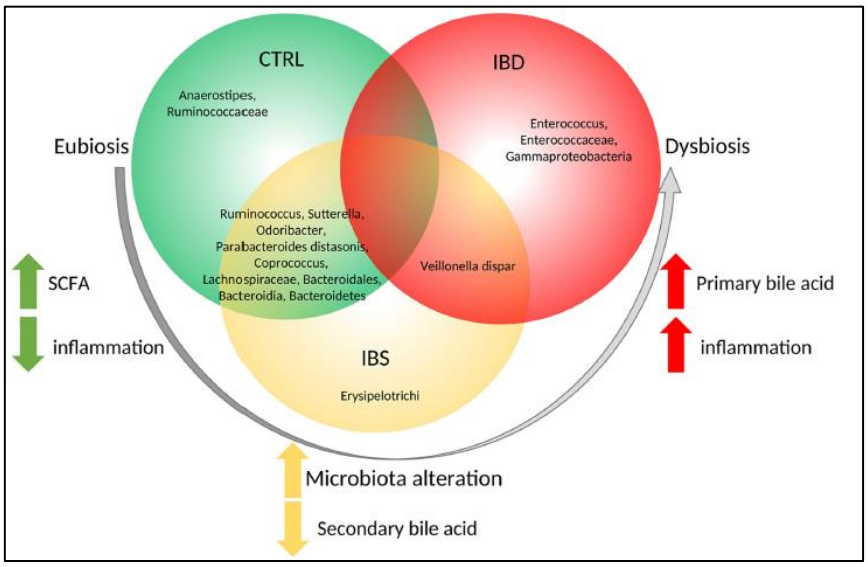


Figure 9: Descriptive model of microbiota composition and its role starting from eubiosis to dysbiosis based on biopsy samples (32).

3.2 JUSTIFICATION

IBD is a lifelong disease, particularly frequent in early adulthood, with intermittent periods of relapsing and remission activity. This leads to a marked decrease in the quality of life of the person and the need for periodic controls, chronic treatment and sporadic hospitalizations when the medication is not effective enough.

The incidence and prevalence of IBD is currently increasing, especially among the young ones. There is no direct known cause able to explain this fact, although, it has been highlighted the importance in the changes of gut microbiota and the influence of external environmental causes such as urban areas and pollution.

The management of these patients is complex, there are not an ideal drug or strategy able to cure the disease, neither to be 100% effective. The main goal is to accomplish an extended period of disease remission, which is, without any signs of inflammatory activity, defined as an absence of symptoms (physicians' global assessment) normalization of analytical parameters (CRP <10mg/l, ESR<20mmh, platelet count $<450x10^{12}$/l, WCC $<11x10^{9}$), no need of corticosteroids in the last 12 months, CDAI ≤150/UCAI ≤3 and no alteration in FCS (macroscopically normal-appearing mucosa). If all these items are fulfilled, we talk about quiescent-IBD (176). Nevertheless, in about 35-39% of these patients will appear symptoms compatible with IBS diagnosis, a higher ratio compared with the one found in general Western populations (estimated between 10-20%, 5-10% in the most recent series using Rome IV criteria)(2)(32). Whether this event is due to a relapse of the disease, intestinal changes induced by the chronical inflammation or an overlapping between a true IBS and IBD it is not well-stablished yet. Therefore, this issue takes us to our current topic.

Nowadays, there is no non-invasive test able to differentiate between the two entities. It is true, that there are biomarkers used to detect IBD inflammatory activity and relapse, the most known one being faecal calprotectin. The main issue with this biomarker is its limitations. Whether it has a high NPV, which allows to rule out the diagnosis of IBD if the result of the test is negative, its PPV is lower, and in case of getting a positive result that does not confirm, without the need of performing other tests, the IBD condition (87). Furthermore, faecal calprotectin excludes IBD diagnosis but does not confirm IBS diagnosis. Therefore, according to the results of this biomarker we can see two types of patients: the ones with high levels of calprotectin suggesting IBD but not confirmed until the performance of a colonoscopy, and the ones with negative values with a clinical suspicion of IBS because of exclusion.

With all the information given, the problem arises by itself, what happens with these patients? What about the ones with known quiescent IBD, IBS-like symptoms, without a clear diagnosis between both diseases?

With the current available techniques, the best method to achieve the definitive diagnosis and to differentiate between both entities is to conduct a FCS, an invasive and expensive procedure, with a low convenience trait for the patient, not exempt of complications, such as risk of perforation, bleeding or infections.

Considering the statement presented above, it is logical to affirm that there is a need to introduce a new diagnostic tool capable of differentiating between IBS and IBD.

In this project, the proposed one which can face the suggested challenge is RAID-Dx. This tool is based on the determination of 10 different phyla of bacteria residing in the gut in order to find out the microbiological footprint of the patient and compare it with the microbiota signature of IBS and IBD, each of them presenting a different population proportion and predominance. By doing so, we may be able to optimally categorize and treat patients with quiescent IBD and IBS-like symptoms without the need of performing a colonoscopy.

4.

HYPOTHESIS

The proposed project is based on the following hypothesis: gut microbiota composition is different in individuals with IBS-like symptoms suffering from true IBS compare to the ones with quiescent IBD. Therefore, a test based on the detection of bacterial dysbiosis (such as RAID-Dx) must be a good diagnostic tool capable to differentiate between the two entities.

5.

OBJECTIVE

5.1 MAIN OBJECTIVE

The main purpose of the study is to find out if RAID-Dx, a non-invasive test based on the disruption of the population of gut microbiota, is an optimal tool to differentiate true irritable bowel syndrome forms from IBD flare-up in quiescent IBD patients with IBS-like symptoms. For that reason, it will be necessary to determine its sensitivity, specificity and predictive values for the detection of both, IBS and IBD, in qIBD patients with IBS-like symptoms.

5.2 SECONDARY OBJECTIVES

1 – To detect if it exists a difference in the microbiological footprint in the patients regarding to belong in the same group with the same disease.

2 – To assess the interaction of dietary habits in RAID-Dx results and accuracy.

3 – To calculate the prevalence of IBS in qIBD patients with IBS-like symptoms.

6.

MATERIAL AND METHODS

6.1 STUDY DESIGN

The proposed study has a cross-sectional or transversal design. It will be a multicentric study conducted in 6 centres of Girona (Hospital Doctor Josep Trueta, Hospital de Santa Caterina, CAP Montilivi, CAP Can Gibert del Pla), Figueres (Hospital de Figueres) and Olot (Hospital d'Olot i Comarcal de la Garrotxa).

6.2 TARGET POPULATION

The target population of the study are the patients with IBS-like symptoms meeting criteria Roma IV attending to the consultation, diagnosed with inflammatory bowel disease in a quiescent state, this is, without clinical, radiological nor endoscopic evidence of active illness during ≥6 months, living in the sanitary regions of the centres mentioned above.

'Remission', or quiescent state, will be defined as one or more of the following for ≥6 months prior to the index appointment:

1. Mayo Index Score <1 (ulcerative colitis) or SEIS-CD Index Score ≤2 (Crohn's disease).
2. No change in IBD medications with no new symptoms reported.
3. Physician documentation of remission status (UC-DAI and CDAI).

6.2.1 Inclusion criteria

- Age between 18 and 50 years.
- Scheduled colonoscopy in the following 2 months.
- Patients with IBS-like symptoms with a known quiescent-IBD diagnosis meeting Rome IV criteria.
- No need for treatment intensification in the last 6 months, as well as no modification of the treatment before the colonoscopy.
- Ability to understand the study procedure.
- Accepted and signed informed consent.

6.2.2 Exclusion criteria

- Evidence of antibiotic consume 1 month before the sample obtention.
- Use of probiotics and/or prebiotics one month before the sample obtention.
- Reported recent diagnosis (in the previous 3 months) of bacterial, fungi or parasitic infections of the gastrointestinal tract.
- Use of non-steroidal anti-inflammatory drugs in the previous 3 months.
- Use of PPIs drugs in the previous 3 months.
- Use of thiopurines as IBD maintenance treatment.
- Continuous treatment with steroids.
- Substance abuse or dependency (except for caffeine and tabaco). Excessive alcohol intake will be considered 40g/day for women and 80g/day for men.
- Serious comorbidity clinically assessed.
- Colectomy.
- Pregnancy or lactation.
- Evidence of active IBD assessed by clinical indices (CDAI for CD and UC-DAI for UC), endoscopic evaluation or imaging.

6.2.3 Individual exclusion from the study

Once patients have accepted their participation into the study, they can choose to leave it at any point of its course. If, by any chance, a subject decides to not continue with their participation in the project, the data collected from them will not be used. Nevertheless, a member of the research team will contact the participant in order to assess their reason to quit the study. By doing so, it could be analysed the characteristics of the patient who leave the project and evidence, at the end of the research, a possible selection bias, in case of many patients with similar characteristics decide to not participate anymore in the study.

6.3 SAMPLING

6.3.1 Sample size

The main goal for the studied test is to have a high PPV, which will allow to detect the patients suffering from true IBS who do not need a colonoscopy. Nevertheless, it is not clear the real prevalence of IBS among qIBD patients, some studies have shown a mean prevalence of 35%, but it could be higher than that (146). Moreover, EPIDAT program, the one used to estimate sample size in diagnostic test studies, it does not allow to calculate it giving PPV as the model measurement.

For that reason, a proportion of population estimation has been performed instead of other type of size sample calculation, using the GRANMO free application.

It has been assumed an expected proportion of 0.85 with an α of 5% and a confidence level of 95%. It has been anticipated a replacement rate of 5%. By doing so, the number of participants needed for conducting the study is calculated to be **207**.

6.3.2 Sample selection and enrolment

The sampling method selected for the study will be a non-probabilistic consecutive one. Known patients from the Digestive Department of the enrolled centres, referred above, attending to its outpatient clinics will be selected according to the inclusion and exclusion criteria. Once there, potential candidates will be informed about the study, its goal and its voluntary participation will. Their anonymity will be respect through all the study course, as it is reflected in the informed consent (Annex 8), which must be signed by the participant.

The sampling collection will be conducted until the sample size is correctly achieved.

6.3.3 Estimated time of recruitment

The estimated time of patient recruitment will be 7 months. The sample analysis will begin once the patient signs the informed consent, accepting their participation in the study, and the stool sample is obtained. The time of recruitment is an estimation, it will vary according to the participation rate and sample obtention during the first months of the study.

6.3.4 Response rate assurance

In order to prevent the discontinuation of participants a detailed information sheet about the study has been made which will be given together with the informed consent. The team members (doctors) in charge of selecting and receiving the patients who are willing to participate in the study, are going to give a brief and simple explanation about the study plan, apart from the documents mentioned before, and transmit a feeling of assurance to the potential participants in order to completely enrol them in to the study. By doing so, the research team trust to keep a response rate superior to 80%.

6.4 VARIABLES AND MEASUREMENTS

6.4.1 Principal variables

Independent variable: RAID-Dx test

The independent variable of the study is the application of RAID-Dx test in patients with IBS-like symptoms, which is, the detection of alterations in ten microbiota populations

(including species, phyla and families) based on the analysis of 16S rRNA (mainly) of its microorganisms.

RAID-Dx is a test *in vitro* based on the analysis of the gut microbiota developed for the diagnosis of IBS (diagnosis in positive). Furthermore, it allows to differentiate between IBS and IBD. The only thing required is a stool sample, from where the microbiome DNA is going to be extracted, amplified through qPCR technique and analysed, in order to assess the relative abundance of the different, pre-selected, bacterial populations (including families and phyla, not only specific species).

The stool sample is going to be collected by the patient itself in their homes or in the moment of clinical consultation. The preferred scenario is the last one, because the sample must be kept in sterilize conditions and frozen before 2 hours of its obtention. In any case, stool samples must be collected before starting colonoscopy preparation. Once obtained, the samples will be labelled and stored at -20°C freezer by the research group.

The microbiome analysis will be performed by specialized Microbiological Service and will take place in a central lab at *Parc Científic I Tecnològic de la Universitat de Girona (GoodGut SL)*. The DNA will be obtained using a specific kit for soil samples (the one which have shown greatest results). Then, it will be amplified by qPCR method, with positive (patterns with a known concentration of the studied gene) and negative controls in order to assure the correct run functioning. The genetic material obtained will correspond to bacterial 16S rRNA, mainly.

Once obtaining the results, a pre-design and validated algorithm is going to be used in order to differentiate between the groups of interest and achieve the diagnosis. Thanks to the RAID-Dx software a result of IBS positive versus negative is going to be achieved.

RAID-Dx was tested in a previous study. It was a concept-prove study with a total of 163 patients, 52 patients diagnosed with IBS, 52 with IBD and 61 healthy individuals (controls). The test showed a sensibility of 88’2% and a specificity of 85’7% for the positive diagnosis of IBS patients, and 88’2% and 88’6% of sensitivity and specificity respectively for IBD diagnosis as well.

This is a dichotomous qualitive variable and it will be defined according to the presence or not of either IBS or IBD. The study groups, then, will be the following ones:

- Patients with IBS and IBD negative results (group 0)
- Patients with IBS positive diagnosis and IBD negative result (group 1).
- Patients with IBS negative result and IBD positive diagnosis (group 2).
- Patients with unclear or not calculable result (group 3).

<u>Dependent variable: outcome</u>

The optimal differentiation between reactivation of IBD and “true IBS” in patients with functional gastrointestinal symptoms.

As explained above, the microbiological analysis will be performed using RAID-Dx test by a specialized Microbiological Service from GoodGut at *Parc Científic i Tecnològic of Girona University*.

The RAID-Dx results are going to be correlated trough the current gold standard technique for the diagnosis of intestinal inflammation, which is, a colonoscopy.

The colonoscopy is going to be performed in the Gastroenterology Service of one of the hospitals adhered to the study scheduled previously to the start of the IBS-like symptoms.

According to colonoscopy results, two different groups will be identified:

- Group A: negative colonoscopy, which is, no endoscopic signs of inflammation or IBD reactivation.
- Group B: positive colonoscopy, which is, endoscopic signs of inflammation compatible of IBD reactivation (according to Mayo and SEIS-CD scores).

6.4.2 Secondary variables

Diet

Dietary habits represent a potential factor of interaction. Because of that, it may play a role in RAID-Dx test results, and it is going to be considered in the statistical analysis in order to assess if it represents an interaction factor.

Diet is going to be assessed through a validated food frequency questionnaire from PREDIMED study from Navarra University (Annex 7), which is going to be given to all participants and, then, used to stratify them according to macronutrients (g/day), micronutrients (mg/day or µg/day) and total kcal consumed.

6.4.3 Covariables

The variables described in the following lines must be considered when RAID-Dx results are analysed. These covariables may play a role in the modification of the outcome due to their influence over gut microbiota composition.

Type of IBD:

Under IBD name there are two different entities with different behaviour, UC and CD. For that reason, in order to assess the existence of any modification due to the type of IBD, the participants are going to be divided according that: “1” for UC and “2” for CD.

Moreover, regarding CD, disease localization is also important, and it will be assessed trough previous colonoscopies of the own participant. The different results are going to be divided according to the Montreal phenotypic classification of CD: ileocecal (1), colonic (2), ileocolonic (3), upper GI involvement (4).

IBD burden:

It refers to the amount of time since the diagnosis of IBD in the patient. It will be express in months.

IBD remission treatment:

Among the patients with quiescent IBD there are differences in the therapeutic management and step where they are found. According to that, one can find different options to achieve remission: aminosalicylates, thiopurine, methotrexate, anti-TNF or new biological drugs treatments.

Sex:

The gut microbiota can be slightly modified due to hormonal interactions. Moreover, it is known IBS is more prevalence in women than in men. For that reason, gender could influence in the results. It represents a dichotomous qualitive variable and it will be recorded as “F” for women and “M” for men.

BMI:

The body weight and fat proportion are another gut microbiota modifying factors. It is a continuous quantitative variable and it is going to be assessed using BMI system, which is calculated using the following formula: $BMI = \frac{weight}{height^2}$, expressed in kg/m².

Tobacco consumption:

Tobacco is known to be a risk factor for developing CD and a protector in relation with UC. Moreover, it can modify the gut microbiota balance (177). The consumption will be assessed using a yes/no question, plus the option of ex-smoker, and, in case of affirmative answer, the consumptions is going to be recorded with the measurement of cigarettes per year.

Alcohol consumption:

There is evidence of a strong negative influence of alcohol dependency on microbiome (178). It will be assessed as grams of alcohol consumed per day (continuous quantitative variable).

Consume of caffeine:

Recorded as yes/no (dichotomous qualitative variable).

Concomitant treatments:

All the medications consumed by the participants will be reported to assess the possible interactions with the gut microbiota. As mentioned before, antibiotics, NSAIDs, PPIs and steroids, will be an exclusion criterion if they have consumed one month before the sample obtention. This data will be not considered in the statistical analysis.

7.

DATA COLLECTION

7.1 SAMPLE OBTENTION

Stool samples are going to be collected in the outpatient consult or at home. They will be split in two different circuits: one part for the study, used for DNA extraction and RAID-Dx application, another for a routine analysis of faecal calprotectin, the detection of *Clostridium difficile* toxin and the realization of a stool culture in order to rule out any parasitical infection.

The stool samples are going to be collected, if possible, on the day of the consultation. By any chance of not being able to gather it then, the sample must be obtained before the colonoscopy preparation, because the substances used in it would modify the gut microbiota.

Once the samples are collected, they must be kept in a sterile container and preserved at -20 degrees before 2 hours from their obtention.

As for RAID-Dx analysis, DNA is going to be extracted from the samples following GoodGut SL. protocol. Once obtained, quantitative PCRs are going to be performed and the results analysed, following, as well, the GoodGut SL stablished protocols. Finally, the genetic material collected will undergo through the RAID-Dx algorithm and the classification of IBS/IBD, and positive/negative according to it, will be done.

7.2 COLONOSCOPY

Before the execution of the colonoscopy two conditions must be achieve:

- Stool sample obtention.
- Undergo to an adequate bowel preparation to ensure a proper examination of the totality of the mucosa.

The colon preparation can be done using different compounds and following the manufacturer's guidelines, such as polyethylenglicol substances, biphosphate based or sodium sulphate with oxide magnesium.

Once the two conditions mentioned above are fulfilled the colonoscopy can be performed. The endoscopists in charge of the assessment are going to perform a

thorough inspection in order to detect any stigma of inflammation, from the rectum to the ileocecal valve, in case of Crohn's disease to the terminal part of the ileum if possible.

Colonoscopy evaluation of the activity of the disease is going to be based on the endoscopic index of both UC and CD, Mayo and SEIS-CD index scores respectively.

Apart from the endoscopic inspection, biopsies are going to be taken in order to assess the presence of CMV infection, even if there are no evident ulcers.

7.3 BLINDING

Both, participants and physicians, will be aware of the application of the test. Nevertheless, the person in charge of the sample analysis (RAID-Dx) won't know the results of the colonoscopy, in order to avoid the influence by the endoscopic diagnosis.

7.4 EFFICIENCY AND SAFETY OF THE TESTS AND PROCEDURES

RAID-Dx uses a stool sample in order to extract the microbiota DNA and to perform an amplification and analysis of selected bacteriological populations. For that reason, there are no major risks associated with the collection of stool sample.

Colonoscopy is an invasive test and it has associated risks that one must consider. Nevertheless, in this study the selected patients will undergo through a colonoscopy which has been scheduled previously to the study (inclusion criteria). For that reason, the participation in it does not confer an increased risk compared to the patients not taking part on the study. The main complications related to the realization of a colonoscopy include:

- Bleeding.
- Colonic/rectum perforation.
- Diverticulitis.
- Adverse effects from sedation, mainly as cardiovascular complications.

7.5 ASSESSMENT OF DATA QUALITY

In order to assess the quality of the diagnostic test used in the study, repetitive measurements will be done in order to control it. RAID-Dx test will be applied in the same stool sample every 20 patients, randomly, in order to achieve that purpose.

7.6 STUDY CIRCUIT

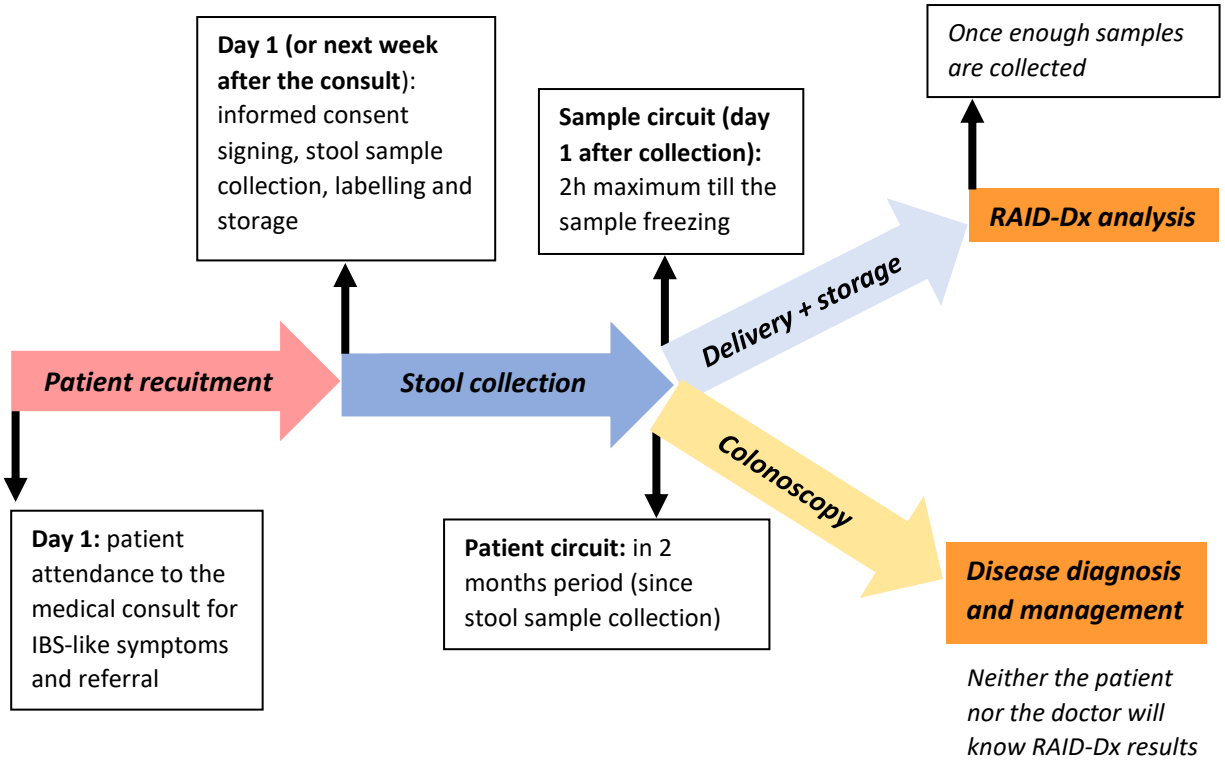
The study will require a coordinated task among all members involved in the research. The data collection will follow the suggested circuit:

1. Recruitment: the personnel from Gastroenterology Department, as well, as PHC centres will be responsible for the patient recruitment task, identify the patients who meet the inclusion criteria, enrol them by proposing and informing about the aim of the study and the benefits that may lead in the future. They will supply to the patients the document regarding the information about the content and phases of the study and, once signed, the informed consent (Annex 8).

2. Sample obtention: stool samples will be collected in the medical office or, if not possible, patients will gather it up at home and then bring it to the reference centre in less than 2 hours. The stool sample will always be obtained before the beginning of colonoscopy preparation. Once the sample is collected, it will be labelled and stored, the patients will be asked to fill a data collection sheet containing personal and clinical information in order to describe and analyse the participant characteristics, completed with data from their clinical record.

3. Sample processing: samples must be kept at -20°C from the moment they are collected, maximum from 2 hours of its collection. Once stored, samples will be sent to GoodGut SL in *Parc Científic I Tecnològic de Girona*, where they are going to be analysed and processed for RAID-Dx application by the microbiological researchers.

4. Colonoscopy: after the sample collection a colonoscopy will be performed. Endoscopist of the Digestive Department participating centres will be informed, previously to the colonoscopy, about the participation of the patient in the study, and a whole and carefully inspection, with biopsy intake, will be done. The results of the colonoscopy are going to be kept from the microbiological researchers in order to avoid any influence in their judgement of RAID-Dx results. Even though, the physicians responsible of the patient (first step of the circuit) do will be informed of the results of the colonoscopy, in order to perform the appropriate management of the patient situation without waiting until the ending of the study.


Day 1 (or next week after the consult): informed consent signing, stool sample collection, labelling and storage
Sample circuit (day 1 after collection): 2h maximum till the sample freezing
Once enough samples are collected
RAID-Dx analysis
Delivery + storage
Patient recuitment
Stool collection
Colonoscopy
Day 1: patient attendance to the medical consult for IBS-like symptoms and referral
Patient circuit: in 2 months period (since stool sample collection)
Disease diagnosis and management
Neither the patient nor the doctor will know RAID-Dx results

8.

STATISTICAL ANALYSIS

8.1 UNIVARIATE DESCRIPTIVE ANALYSIS

The variables will be expressed as percentages for qualitive or categorical variables (IBD type, remission treatment used, sex…). On the other hand, quantitative or continuous variables (age, BMI…), if they are not normally distributed, will be expressed as a median (1^{st} quartile – 3^{rd} quartile). In case that they follow a normal distribution, arithmetic mean and standard deviation (± SD) will be calculated.

8.2 BIVARIATE ANALYSIS

The primary end point of the study is to assess the usefulness of RAID-Dx test to differentiate IBD patients from IBS among individuals with a known IBD in quiescent state. The nomenclature used will be IBD positive/negative and IBS positive/negative in every patient. In order to achieve that, once obtained the samples needed to apply the mentioned test, a colonoscopy will be performed in order to assess the inflammatory status of the gut in every patient included in the study, which is the gold standard test until the moment to differentiate between true IBS and IBD recrudescence. To determine differences between various groups of RAID-Dx results unpaired Student's t-test is going to be used for normally distributed variables (disease location, age, gender…) and Mann-Whitney U-test for non-normally distributed ones (IBD type, e.g.).

Furthermore, in order to evaluate the validity of the test we will need to calculate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios (positive and negative), associated with RAID-Dx and its ability to diagnose either IBS and IBD in the patients who meet the inclusion criteria. A curve ROC will be represented for RAID-Dx results, and Harrell's C index will be used to assess the area under the curve (to compare predictive powers of survival models).
As the importance of the test's positive predictive value, and to accomplish one of the study's secondary objectives, we will, as well, calculate the data about IBS prevalence in qIBD patients with IBS-like, dividing positive individuals (detected by colonoscopy) per all the individuals of the sample, using a calculation with a 95% of confidence intervals.

In order to analyse the interaction between diet and RAID-Dx result, the same analysis is going to be done but stratified according to the type of diet.

Statistical significance will be set at *p* value <0’05.

9.

ETHICAL CONSIDERATIONS

9.1 ETHICAL GUIDELINES

The research will respect the fundamental principles of the Helsinki Declaration for medical research involving human subjects (World Medical Association), will follow all the aspects covered in the Council of Europe and the Convention on Human Rights and Biomedicine as well as the requirements established by the Spanish and Catalan legislation in the field of biomedical research, data protection and bioethics.

9.2 LEGAL ASPECTS

All participants will sign informed consent, compliant with Data protection law [LO 3/2018, of December 5th of 2018 on *Protection of personal data and digital rights guarantee (LOPDGDD)* and published on December 6th, 2018]. The institutional review board and ethical committee will approve the present research project to take place in the study centre.

9.3 AUTONOMY, PRIVACY AND CONFIDENTIALITY

Informed consent must be obtained from each subject (Annex 8) after full explanation of the purpose and nature of all procedures used. In order to carry out this study it will be necessary to present the research protocol to the Clinical research Ethics Committee (CEIC) of Hospital Universitari Josep Trueta.

Moreover, participants have the right, and they will be informed accordingly, to access, modify, oppose or remove their personal data.

9.4 SAFETY CONCERNS

Related with the tests

RAID-Dx is a secure non-invasive test with the only requirement of a stool sample. For that reason, there are no safety concerns regarding the studied test.

9.5 MANAGEMENT OF THE EXCESS SAMPLE

Final sample storage will be performed within -20ºC GoodGut Biobank freezers, which is included in the Biobank national network, for future usage when considered and approved by the participant, who previously would have firmed the informed consent related to biobank preservation samples. If the participant decides to not firm the informed consent the sample will be destroyed.

9.6 TRANSPARENCY

Procedures to inform about any digression in the main study planification

If, by any chance, the initial planning of the project suffers a deviation in its course once begun, the research team will contact the participants in order to inform them about the new situation and project and check their desire to stay in the study or leave it. If they choose to keep participating in it a new signed informed consent, including any changes from the initial project, is going to be required.

Conflict of interests

All the investigators will have to declare no conflicts of interest. The authors, as well, will declare the main goal of this research is to develop generalizable knowledge to improve human health and quality of life.

10.

CHRONOGRAM AND WORK PLAN

10.1 Participating centres

The following table shows the participating centres in the study:

Participating centers
Hospital Universitari Doctor Josep Trueta (Girona)
Hospital Santa Caterina (Girona)
CAP Can Gibert del Pla (Girona)
CAP Montilivi (Girona)
Hospital de Figueres (Figueres)
Hospital d'Olot i Comarcal de la Garrotxa (Olot)

10.2 Research team personnel

The research team will be composed by a multidisciplinary team composed by a head-researcher from the GoodGut group from Girona (H-R), 7 physicians from the centres mentioned above, both gastroenterologists and primary health doctors (D), and 2 microbiologists from GoodGut group (Mb-R). In addition, the team personnel will include a statistician (S) in order to perform the data monitoring and the statistical analysis, as well as the interpretation of the results jointly with the rest of the team.

SI AL FINAL ES DECIDEIX QUE SÍ AFEGIR INFERMER/A!

10.3 Project stages

Phase 1: preparation and coordination of the project. Estimated time: 5 months.

In the first stage of the project are included:

1. Bibliography research related to the topics of the study.

2. The selection and participation proposal to the centres included in the project. It will be needed a written permission to perform the study by the executive department of each hospital. The principal researcher is responsible of this task
3. Protocol elaboration. One person of the team is going to be responsible of writing the protocol, but all members of the study must agree with the procedure.
4. The presentation of the project to the Ethical Committee of Hospital Josep Trueta and, of course, its approval.
5. The first meeting of the whole team: once the protocol is approved all the research team members will need to attend to a meeting with the goal of informing and coordinating the professionals participating in the study. The team meeting must be done prior to the start of the patient recruitment. The head researcher will explain the information regarding the project and assign the different tasks. Furthermore, the research team will discuss the practical issues and adapt the original work plan to hospital's needs.

The time requested in this stage is estimated to be 5 months. Although, it may vary according to the time taken by Ethical Committee to approve the project.

Phase 2: data collection and field work. Estimated time: 12 months.

The second stage is where the enrolment of the participants and the stool sample collection, processing and analysis will be done. Therefore, this is the longest phase of the study.

6. Patient recruitment: the doctors participating in the project will consecutively recruit patients applying the inclusion and exclusion criteria. Note that sample selection can be extended until the required size is achieved.
7. Sample collection and final enrolment of the participants: stool sample collection, food frequency questionnaire and anthropometric measurements.
8. Laboratory processing and RAID-Dx testing service: once having enough samples collected, the RAID-Dx test will be performed. The average duration of RAID-Dx analysis is 2 days, therefore the extension of this point will only be justified by waiting the collection of enough number of samples to apply the test.

Phase 3: data analysis and interpretation. Estimated time: 4 months.

9. Statistical analysis and interpretation of the results: analysis of data by the subcontracted service (statistician). It will begin once there be enough RAID-Dx test results and its pertinent colonoscopies performed, and the final analysis will be carried out at the end of all data collection.

10. Second research team meeting: this time there will not be reunited all team members. Head researcher together with microbiologist and one of the gastroenterologists of the team will discuss and interpret the results.
11. Final report elaboration: the main researcher together with assessment of other team members will write a report with the relevant aspects of the study.

Phase 4: publication and dissemination. Estimated time: 7 months.

This will be a long stage, as well as the second one, because the need of the study to be accepted by medical journals and national congresses. It may be many different factors modifying the time of this stage, most of them not depending on the researchers of this study.

- Publication in reviews: writing a journal article. Application to different reviews in order to publish the findings. The responsible for this task will be the head-researcher.
- Attendance to two national congresses, *Societat Catalana de Digestologia* and *Asociación Española de Gastroenterología* to present the conclusions of the study.

TASKS	Person	2019		2020												2021								
		Nv	De	Ja	Fe	Mr	Ap	My	Jn	Jl	Au	Se	Oc	Nv	Dc	Ja	Fe	Mr	Ap	My	Jn	Jl	Au	Se
PHASE 1: PROJECT PREPARATION AND COORDINATION																								
Bibliography research	H-R																							
Centres selection and proposal	H-R																							
Protocol elaboration and approval	H-R																							
Coordination of the research team: team meeting	All members																							
PHASE 2: FIELD WORK AND DATA COLLECTION																								
Patients recruitment	D																							
Sample collection	D																							
Probe design in the laboratory	Mb-R																							
Setting up of RAID-Dx: DNA extraction and amplification	Mb-R																							
Setting up of RAID-Dx: qPCR analysis	Mb-R																							
Setting up of RAID-Dx: data processing	Mb-R																							

PHASE 3: DATA ANALYSIS AND INTERPRETATION																							
Statistical analysis	Statistician																						
Analysis and interpretation of results	S + Mb-R																						
Research team meeting	All members																						
Final report elaboration	H-R																						
PHASE 4: PUBLICATION AND DISSEMINATION																							
Divulgation: publication in reviews	H-R + D																						
Results dissemination (congresses)	H-R + D																						

11.

BUDGET

11.1 NOT-INCLUDED COSTS

Staff: the personnel participating in the research team will not be extra rewarded for their involvement in the project. By doing this, we are trying to avoid any economic grounds be the main incentive to join in the study. Researchers will be rewarded by the scientific prestige among the medical community.

Available materials: patients with quiescent IBD suffering from IBS-like symptoms are going to undergo through a colonoscopy whether they are included in the study or not. For that reason, the costs related to the realization of the colonoscopy will not be included in this study.

11.2 INCLUDED COSTS

Material costs

Printing costs: study information sheet, informed consents and diet formulary are required to be printed for every participant. The estimated printing cost for each paper is 0'05€/page.

Stool containers: one for each participant. Cost: 2'5€/container.

RAID-Dx testing service: 185€ for each patient.

Subcontracted services

Statistician: the subcontracted statistical analysis service is going to receive a salary of 35€/h and is going to be required a total amount of 72h, approximately.

Allowances and meal costs

Team meetings: during the study it will be a total of 2 meetings, the first involving all members of the team, the second fourth of them. The budget included here refers to

the cost of the meal and allowance of each person, which is 65 euros per person per meeting.

Divulgation costs

Publication costs: the results of the study are expected to be published in a journal article, and we will assume a total cost related to publication fees of 1500-2000euros.

National congresses: some of the team members will attend to national congresses to disseminate the results. We include the costs related to the attendance to the *Societat Catalana de Digestologia* and *Associación Española de Gastroenterología* congresses, which are 600€ each per attendant. There will be going a total of two research members in every congress, which is a total amount of 2400€.

EXPENSES	COST PER UNIT	NUMBER OF UNITS	SUBTOTAL
Material costs			
Printing costs	0'05€/page	1656	82'8€
Stool containers	2'5€/container	207	517'5€
RAID-Dx testing service	185€	207	38295€
Subcontracted services			
Statistical analysis	35€/h	72h	2520€
Allowances and meal costs			
Team members allowance	35€/reunion	10 x 1 meeting 4 x 1 meeting	490€
Meals	30€/reunion	10 x 2 meeting 4 x 1 meeting	420€
Divulgation costs			
Article publishing fees	2000€/publication	1 publication	2000€
Inscription to national congresses	600€/congress per attendant	2 congresses; 4 inscriptions	2400€
TOTAL			**46725'3€**

12.

STUDY LIMITATIONS

The present study has several limitations that should be acknowledge:

1. The study uses a non-probabilistic sampling method, for that reason, the subjects of the studied population do not have the same chance to be elected to constitute the sample. Even though, the consecutive method is one of the non-probabilistic methods that induces less bias, as theoretically guarantees a free-of-choices selection. However, it remains possible the fact professionals responsible of participants recruitment do not suggest the study at every potential candidate, generating a selection bias.

2. Both, the reference test and the test being evaluated, should be applied and interpreted blindly and independently, to avoid diagnostic suspicion bias. Nevertheless, the ethics behind the project require the physician to know the results of the colonoscopy in order to provide the appropriate treatment for the resulting disease. Therefore, either the doctors and the participants will know the result of the gold-standard and the study will be defined as single-blinded, with the personnel in charge to process and analyse RAID-Dx results being the only ones to not know the real condition of the patient. By doing so, we will try to diminish the influence towards the researchers of knowing the results of the colonoscopy before analysing the stool samples and applying the RAID-Dx test.

3. To decrease interobserver variability, tests will be performed and interpreted by just one expertise, who will be responsible of the processing and application of RAID-Dx tests for every stool sample.

4. The main enemy of observational studies are confusion variables. In order to minimize that fact and try to avoid them, detected covariables will be included in the statistical analysis to detect any potential interaction with the dependent variable (RAID-Dx test result).

5. In the models of study like the one proposed, one potential bias is the so-called spectrum bias, which refers to the inclusion of the patients with serious symptoms/disease and put aside the ones with mild to moderate symptoms, mainly because of the lack of clinical help searching. This fact is needed to take in account and assessed in the initial enrolment phase, in order to detect and analyse it in the statistical part later.

6. Another limitation of the study is the lack of experience regarding RAID-Dx test, as a new diagnostic tool recently introduced to the market. Therefore, data about post-marketing release is still needed.

7. In this study, the PPV has been set at 80% ± 5% of accuracy. Therefore, 1-2 every 10 patients will not obtain a positive result for IBS and undergo through a colonoscopy unnecessarily. Nevertheless, 8-9 patients will spare the time and inconvenience of it.

8. There is a lack of studies regarding the modification of gut microbiota whether one drug or other is used. It seems thiopurines may induce a decrease in the diversity of gut microbiota, more than IBD itself (35). Because of this fact, RAID-Dx test results may be modified in the patients treated with the mentioned drugs. To try to avoid that, the use of thiopurines in maintenance therapy has been defined as an exclusion criterion.

9. It is worth mentioning the importance of the colonoscopy in IBD patients. This study is not trying to supress colonoscopy as a diagnostic and following tool in these patients, its aim is to reduce the performance of unnecessary colonoscopies in patients who may not require it. Although, it must be clear that if a person gets an IBD positive result through RAID-Dx test the next step will be the realization of a colonoscopy, as well as scheduled ones in order to do the follow up, due to the increase risk of these patient to suffer a colorectal cancer.

13.

IMPACT ON THE NATIONAL HEALTH SYSTEM AND FUTURE RESEARCH

Gut microbiota has centred an important part of medical spotlights when talking about intestinal diseases, among others. Its role as a diagnostic and therapeutic tool is becoming clearer every day, and future researches about the topic are going to keep increasing. An evident example is this same study.

What is the relevance behind RAID-Dx potential?

If the test proves to have enough positive predictive value to compete with the colonoscopy, the implications following that will have a significant impact in the quality of life of patients suffering from IBD, as well as for the health system itself.

Can you imagine, every time you suffer from abdominal discomfort and diarrhoea, for example, undergo a colonoscopy? What if this situation repeats a mean of 3 times per year?

The possibility to use an alternative test, not invasive and cheaper, in order to achieve the desired diagnosis, will save, the patient and the system, the realization of many unnecessary colonoscopies.

By doing so, IBS and IBD flare-up differentiation could be assessed from a simple stool sample, without the need of the preparation, sedation and complications related to the colonoscopy. Moreover, not only the patient will get benefits from RAID-Dx implementation, but the system as well. It is cheaper than the colonoscopy (185 euros against around 500 euros depending on the centre) and, even more important, it will mean a decompression of endoscopy waiting lists.

Nevertheless, it is important to clarify that the aim of the study, as well as the RAID-Dx development, is not to replace or supress the execution of colonoscopies, which are an excellence, sometimes life-savour, diagnostic tool. Although, one can try to ask anyone who have been through one if they prefer to repeat the experience or collect a stool sample in order to achieve the same information.

Future research

RAID-Dx has been tested in adult population for the diagnosis of IBS and IBD. No studies have been done in paediatric population. Although, the increase in IBD diagnosis and the trend of not using invasive procedures in this group causes a need for researching whether RAID-Dx may be a potential diagnostic tool for paediatric population or not. Although, *Sipponen and Kolho* reported the possibility of FC use to differ adult from childhood disease, indicating a different pattern in the disease behaviour and pathogenic components (179), which can difficult the application of RAID-Dx in paediatric population.

Moreover, one of the main, and biggest, challenges will be the detection of an optimal microbiological sign in this group because, as explained in the introduction, gut microbiota achieve stabilization once in adult life, and can vary during childhood due to different factors, such as hormones, immunologic changes and others. For that reason, before trying to use gut microbiota as a diagnostic tool in infants, a more and complete understanding of it in paediatric population must be acquired.

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15. ANNEXES

Annex 1: IBD risk factors

Enviromental factor	**UC**	**CD**
Smoking	P	R
Animal protein	R	R
Dietary fibre	N	R
Tea or coffee	P	P
Low levels of vitamin D	R	R
Breastfeeding	P	P
NSAID	R	R
Antibiotics in childhood	R	R
Oral contraceptives	R	R
Dipeptil peptidase 4-inhibitors	R	N
Vaccination	N	N
Appendicectomy	P	R
Air pollution	R	R

N: non-influence known factor; P: protector factor; R: risk factor; (3,6,46) .

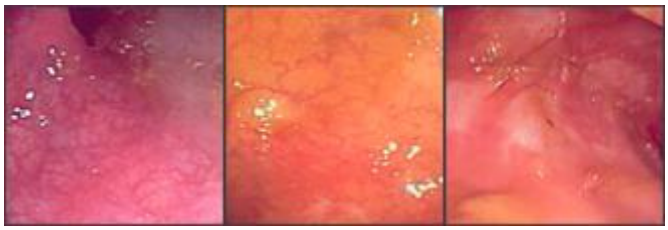
Annex 2: Extraintestinal IBD manifestations

Characteristic	CD	UC	Independent to IBD course
Fever	+++	+	No
Perianal disease*	+++	+	No
Ocular affectation			
*Episcleritis and scleritis**	+++	++	No
Uveitis	+++	+++	No
Skin alterations			
Erythema nodosum	++	+	No
Pyoderma gangrenosum	+/-	++	Yes
Aphthous ulcers/Ulcerous stomatitis	+++	+/-	No
Psoriasis	+	+	Yes
Vitiligo	+	+	Yes
Joint and musculoskeletal manifestations			
Axial arthritis: sacroiliitis and ankylosing spondylitis	++	+	Yes
Peripheral arthritis: *- Type I (polyarticular, big joints involvement, asymmetrical, no joint destruction)* *- Type II (polyarticular, big joints involvement, symmetrical, joint destruction)*	+++ (type I more frequent)	++	No Yes
Hypertrophic osteoarthropathy (rare)	+	+	Yes?
Polymyositis (rare)	+	+	Yes
Hepatopancreatobiliary manifestations			
Primary Sclerosing Cholangitis	+/-	++	Yes
Fatty Liver and cirrhosis	+++	+++	Yes
Gallstones	++ (ileal disease)	-	No
Other extraintestinal manifestations			
Urolithiasis (calcium oxalate)	++	+	No
Bone mass decreasing	++	++	No
Thromboembolic events	+	+	Yes
Reactive amyloidosis	+	+/-	No

*Specially in colonic CD forms (L2)

Annex 3: Mayo endoscopic score

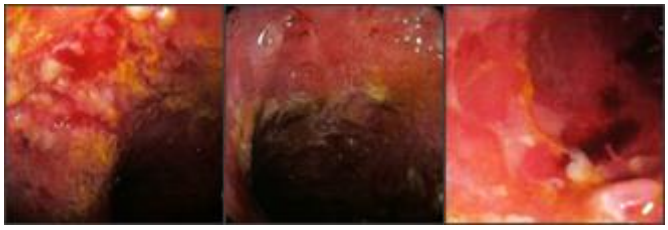
Mayo 0
normal mucosa or inactive disease



Mayo 1
mild activity (erythema, decreased vascular pattern, mild friability)

Mayo 2
moderate activity (marked erythema, lack of vascular pattern, friability, erosions)

Mayo 3
severe activity (spontaneous bleeding, large ulcerations)



From: https://www.igibdscores.it/en/info-mayo-endoscopic.html

Annex 4: SEIS-CD index score

	Ileum	Right colon	Transverse colon	Left colon and sigma	Rectum	Total
Ulcers? 0: no 1: aphthous (0.1-0.5 cm) 2: large (0.5-2 cm) 3: very large (>2 cm)	___+	___+	___+	___+	___=	___+
Surface involved by disease 0: 0% 1: <50% 2: 50-75% 3: >75%	___+	___+	___+	___+	___=	___+
Surface involved by ulcerations 0: 0% 1: <10% 2: 10-30% 3: >30%	___+	___+	___+	___+	___=	___+
Narrowings? 0: No 1: Single, can be passed 2: Multiple, can be passed 3: Cannot be passed	___+	___+	___+	___+	___=	___+
					Grand Total = SES-CD score	

Score decoding:

- 0-2: remission.
- 3-6: mild endoscopic activity.
- 7-15: moderate endoscopic activity.
- >15: severe endoscopic activity.

*A decrease of 50% of the SES-CD score has recently been proposed as prognostically significant.

Annex 5: CDAI AND Mayo scores

CDAI:

	Días	1	2	3	4	5	6	7	Suma x	Factor =	Subtotal
1. Nº heces líquidas o muy blandas		___	___	___	___	___	___	___	_____	2	_____
2. Dolor abdominal (0 = no; 1 = leve; 2 = moderado; 3 = grave)		___	___	___	___	___	___	___	_____	5	_____
3. Estado general (0 = bueno; 1 = regular; 2 = malo; 3 = muy malo; 4 = terrible)		___	___	___	___	___	___	___	_____	7	_____
4. Número de las siguientes manifestaciones clínicas: Artritis/artralgia; Iritis/uveítis; Eritema nodoso/pioderma/aftas; Fisura anal/fístula/absceso; Otras fístulas; Fiebre > 38,5 en la última semana						_____				20	_____
5. Tomando antidiarreicos (0 = no; 1 = sí)						_____				30	_____
6. Masa abdominal (0 = no; 1 = dudosa; 2 = sí)						_____				10	_____
7. Hematocrito. Hombre (47%)/Mujer (42%) - Valor actual						_____				6	_____
8. Peso corporal _____ Peso estándar _____. Porcentaje por debajo del peso estándar						_____				1	_____
										CDAI:	_____

Los apartados 1, 2 y 3 hacen referencia a lo ocurrido al paciente en los 7 días previos a la consulta

Tras la suma se obtendrá una puntuación que corresponderá a:

CDAI < 150 = no activo

CDAI 150-220 = brote leve

CDAI > 220 = brote moderado

CDAI > 450 = brote grave

Mayo partial (without endoscopic assessment) or UC DAI:

Characteristic	**Scores**
Stool frequency	0: normal number of daily stools
	1: 1-2 stools/day more than normal
	2: 3-4 stools/day more than normal
	3: >4 stools/day than normal
Rectal bleeding (indicate the most sever bleeding of the day)	0: none
	1: visible streaks of blood with stool less than half of the time
	2: visible streaks of blood with stool half of the time or more
	3: pure blood passed (alone)*
Global assessment (physician rating of disease activity)	0: normal
	1: mild colitis
	2: moderate colitis
	3: severe colitis
Decoding	<2: remission
	2-4: mild activity
	5-7: moderate activity
	>7: severe activity

*3 points require patients to have ≥50% of bowel motions accompanied by visible blood and at least one bowel motion with blood alone.

Annex 6: Rome IV criteria for IBS

Patient has recurrent abdominal pain (≥1 day per week, on average, in the previous 3 mo), with an onset ≥6 mo before diagnosis

Abdominal pain is associated with at least two of the following three symptoms:

- Pain related to defecation
- Change in frequency of stool
- Change in form (appearance) of stool

Patient has none of the following warning signs:

- Age ≥50 yr, no previous colon cancer screening, and presence of symptoms
- Recent change in bowel habit
- Evidence of overt GI bleeding (i.e., melena or hematochezia)
- Nocturnal pain or passage of stools
- Unintentional weight loss
- Family history of colorectal cancer or inflammatory bowel disease
- Palpable abdominal mass or lymphadenopathy
- Evidence of iron-deficiency anemia on blood testing
- Positive test for fecal occult blood

* The information is from Mearin et al.[1] GI denotes gastrointestinal.

Annex 7: Food Frequency Questionnaire

IDENTIFICACIÓN DEL PARTICIPANTE

NODO
01. Andalucía-Málaga
02. Andalucía-Sevilla-San Pablo
03. Andalucía-Sevilla-V. Rocío
04. Baleares
05. Catalunya-Barna Norte
06. Catalunya-Barna Sur
07. Catalunya-Reus-Tarragona
08. Madrid Norte
09. Madrid Sur
10. Navarra
11. País Vasco
12. Valencia

marque así

así no marque

NODO | CENTRO | MÉDICO | PACIENTE | VISITA

PÁGINA 1

Por favor, marque una única opción para cada alimento.

Para cada alimento, marque el recuadro que indica la frecuencia de consumo **por término medio** durante el **año pasado**. Se trata de tener en cuenta también la variación verano/invierno. Por ejemplo, si toma helados 4 veces/semana sólo durante los 3 meses de verano, el uso promedio al año es 1/semana	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
I. LACTEOS									
1. Leche entera (1 taza, 200 cc)									
2. Leche semidesnatada (1 taza, 200 cc)									
3. Leche descremada (1 taza, 200 cc)									
4. Leche condensada (1 cucharada)									
5. Nata o crema de leche (1/2 taza)									
6. Batidos de leche (1 vaso, 200 cc)									
7. Yogurt entero (1, 125 gr.)									
8. Yogurt descremado (1, 125 gr.)									
9. Petit suisse (1, 55 gr.)									
10. Requesón o cuajada (1/2 taza)									
11. Queso en porciones o cremoso (1, porción 25 gr.)									
12. Otros quesos: curados, semicurados (Manchego, Bola, Emmental...) (50 gr.)									
13. Queso blanco o fresco (Burgos, cabra...) (50 gr.)									
14. Natillas, flan, puding (1, 130 cc)									
15. Helados (1 cucurucho)									

Un plato o ración de 100-150 gr, excepto cuando se indique otra cosa	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
II. HUEVOS, CARNES, PESCADOS									
16. Huevos de gallina (uno)									
17. Pollo o pavo CON piel (1 ración o pieza)									
18. Pollo o pavo SIN piel (1 ración o pieza)									
19. Carne de ternera o vaca (1 ración)									
20. Carne de cerdo (1 ración)									
21. Carne de cordero (1 ración)									
22. Conejo o liebre (1 ración)									
23. Hígado (ternera, cerdo, pollo) (1 ración)									
24. Otras vísceras (sesos, corazón, mollejas) (1 ración)									
25. Jamón serrano o paletilla (1 loncha, 30 gr.)									
26. Jamón York, jamón cocido (1 loncha, 30 gr.)									
27. Carnes procesadas (salchichón, chorizo, morcilla, mortadela, salchichas, butifarra, sobrasada, 50 gr.)									
28. Patés, foie-gras (25 gr.)									
29. Hamburguesa (una, 50 gr.), albóndigas (3 unidades)									
30. Tocino, bacon, panceta (50 gr.)									
31. Pescado blanco: mero, lenguado, besugo, merluza, pescadilla,... (1 plato, pieza o ración)									
32. Pescado azul: sardinas, atún, bonito, caballa, salmón (1 plato, pieza o ración 130 gr.)									
33. Pescados salados: bacalao, salazones (1 ración, 60 gr. en seco)									
34. Ostras, almejas, mejillones y similares (6 unidades)									
35. Calamares, pulpo, chipirones, jibia (sepia) (1 ración, 200 gr.)									
36. Crustáceos: gambas, langostinos, cigalas, etc. (4-5 piezas, 200 gr.)									
37. Pescados y mariscos enlatados al natural (sardinas, anchoas, bonito, atún) (1 lata pequeña o media lata normal, 50 gr.)									
38. Pescados y mariscos en aceite (sardinas, anchoas, bonito, atún) (1 lata pequeña o media lata normal, 50 gr.)									

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Página 2

Por favor, marque una única opción para cada alimento.

	Un plato o ración de 200 grs, excepto cuando se indique	CONSUMO MEDIO DURANTE EL AÑO PASADO								
		NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
			1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
III. VERDURAS Y HORTALIZAS	39. Acelgas, espinacas									
	40. Col, coliflor, brócoles									
	41. Lechuga, endivias, escarola (100 gr.)									
	42. Tomate crudo (1, 150 gr)									
	43. Zanahoria, calabaza (100 gr.)									
	44. Judías verdes									
	45. Berenjenas, calabacines, pepinos									
	46. Pimientos (150 gr.)									
	47. Espárragos									
	48. Gazpacho andaluz (1 vaso, 200 gr.)									
	49. Otras verduras (alcachofa, puerro, cardo, apio)									
	50. Cebolla (media unidad, 50 gr.)									
	51. Ajo (1 diente)									
	52. Perejil, tomillo, laurel, orégano, etc. (una pizca)									
	53. Patatas fritas comerciales (1 bolsa, 50 gr.)									
	54. Patatas fritas caseras (1 ración, 150 gr.)									
	55. Patatas asadas o cocidas									
	56. Setas, níscalos, champiñones									

	Una pieza o ración	CONSUMO MEDIO DURANTE EL AÑO PASADO								
		NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
			1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
IV. FRUTAS	57. Naranja (una), pomelo (una), o mandarinas (dos)									
	58. Plátano (uno)									
	59. Manzana o pera (una)									
	60. Fresas/fresones (6 unidades, 1 plato postre)									
	61. Cerezas, picotas, ciruelas (1 plato de postre)									
	62. Melocotón, albaricoque, nectarina (una)									
	63. Sandía (1 tajada, 200-250 gr.)									
	64. Melón (1 tajada, 200-250 gr.)									
	65. Kiwi (1 unidad, 100 gr.)									
	66. Uvas (un racimo, 1 plato postre)									
	67. Aceitunas (10 unidades)									
	68. Frutas en almíbar o en su jugo (2 unidades)									
	69. Dátiles, higos secos, uvas-pasas, ciruelas-pasas (150 gr.)									
	70. Almendras, cacahuetes, avellanas, pistachos, piñones (30 gr.)									
	71. Nueces (30 gr.)									
	72. ¿Cuántos días a la semana toma fruta como postre?		0	1	2	3	4	5	6	7

	Un plato o ración (150 gr.)	CONSUMO MEDIO DURANTE EL AÑO PASADO								
		NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
			1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
V. LEGUMBRES Y CEREALES	73. Lentejas (1 plato, 150 gr. cocidas)									
	74. Alubias (pintas, blancas o negras) (1 plato, 150 gr. cocidas)									
	75. Garbanzos (1 plato, 150 gr. cocidos)									
	76. Guisantes, habas (1 plato, 150 gr. cocidas)									
	77. Pan blanco, pan de molde (3 rodajas, 75 gr.)									
	78. Pan negro o integral (3 rodajas, 75 gr.)									
	79. Cereales desayuno (30 gr.)									
	80. Cereales integrales: muesli, copos avena, all-bran (30 gr.)									
	81. Arroz blanco (60 gr. en crudo)									
	82. Pasta: fideos, macarrones, espaguetis, otras (60 gr. en crudo)									
	83. Pizza (1 ración, 200 gr.)									

SUMCO 12637-03 (Ret.) 1

marque así

así no marque

NODO	CENTRO	MÉDICO	PACIENTE	VISITA

PÁGINA 3

Por favor, marque una única opción para cada alimento.

	CONSUMO MEDIO DURANTE EL AÑO PASADO								
Una cucharada o porción individual. Para freír, untar, mojar en el pan, para aliñar, o para ensaladas, utiliza en total:	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
VI. ACEITES Y GRASAS									
84. Aceite de oliva (una cucharada sopera)									
85. Aceite de oliva extra virgen (una cucharada sopera)									
86. Aceite de oliva de orujo (una cucharada sopera)									
87. Aceite de maíz (una cucharada sopera)									
88. Aceite de girasol (una cucharada sopera)									
89. Aceite de soja (una cucharada sopera)									
90. Mezcla de los anteriores (una cucharada sopera)									
91. Margarina (porción individual, 12 gr.)									
92. Mantequilla (porción individual, 12 gr.)									
93. Manteca de cerdo (10 gr.)									
94. Marca de aceite de oliva que usa habitualmente:	0 1 2 3 4 5 6 7 8 9 / 0 1 2 3 4 5 6 7 8 9 — No marque aquí								

	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
VII. BOLLERÍA Y PASTELERÍA									
95. Galletas tipo María (4-6 unidades, 50 gr.)									
96. Galletas integrales o de fibra (4-6 unidades, 50 gr.)									
97. Galletas con chocolate (4 unidades, 50 gr.)									
98. Repostería y bizcochos hechos en casa (50 gr.)									
99. Croissant, ensaimada, pastas de té u otra bollería industrial comercial... (uno, 50 gr)									
100. Donuts (uno)									
101. Magdalenas (1-2 unidades)									
102. Pasteles (uno, 50 gr.)									
103. Churros, porras y similares (1 ración, 100 gr.)									
104. Chocolates y bombones (30 gr.)									
105. Cacao en polvo-cacaos solubles (1 cucharada de postre)									
106. Turrón (1/8 de barra, 40 gr.)									
107. Mantecados, mazapán (90 gr.)									

	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
VIII. MISCELÁNEA									
108. Croquetas, buñuelos, empanadillas, precocinados (una)									
109. Sopas y cremas de sobre (1 plato)									
110. Mostaza (una cucharadita de postre)									
111. Mayonesa comercial (1 cucharada sopera = 20 gr.)									
112. Salsa de tomate frito, ketchup (1 cucharadita)									
113. Picante: tabasco, pimienta, pimentón (una pizca)									
114. Sal (una pizca)									
115. Mermeladas (1 cucharadita)									
116. Azúcar (1 cucharadita)									
117. Miel (1 cucharadita)									
118. Snacks distintos de patatas fritas: gusanitos, palomitas, maíz, etc. (1 bolsa, 50 gr.)									
119. Otros alimentos de frecuente consumo:									
119.1									
119.2									
119.3									

SUNICO 12637-03-2

Por favor, marque una única opción para cada alimento.

IX. BEBIDAS

	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
120. Bebidas carbonatadas con azúcar: bebidas con cola, limonadas, tónicas, etc. (1 botellín, 200 cc)									
121. Bebidas carbonatadas bajas en calorías, bebidas light (1 botellín, 200 cc)									
122. Zumo de naranja natural (1 vaso, 200 cc)									
123. Zumos naturales de otras frutas (1 vaso, 200 cc)									
124. Zumos de frutas en botella o enlatados (200 cc)									
125. Café descafeinado (1 taza, 50 cc)									
126. Café (1 taza, 50 cc)									
127. Té (1 taza, 50 cc)									
128. Mosto (100 cc)									
129. Vaso de vino rosado (100 cc)									
130. Vaso de vino moscatel (50 cc)									
131. Vaso de vino tinto joven, del año (100 cc)									
132. Vaso de vino tinto añejo (100 cc)									
133. Vaso de vino blanco (100 cc)									
134. Vaso de cava (100 cc)									
135. Cerveza (1 jarra, 330 cc)									
136. Licores, anís o anisetes... (1 copa, 50 cc)									
137. Destilados: whisky, vodka, ginebra, coñac (1 copa, 50 cc)									

138. ¿A que edad empezó a beber alcohol (vino, cerveza o licores), incluyendo el que toma con las comidas con regularidad (más de siete "bebidas" a la semana)?

Edad (años)

Decena
Unidad

139. ¿Cuántos años ha bebido alcohol con regularidad (más de siete "bebidas" a la semana)?

Años

Decena
Unidad

119. Otros alimentos de frecuente consumo

119.1 (No marque aquí)

119.2 (No marque aquí)

119.3 (No marque aquí)

Si durante el año pasado tomó vitaminas y/o minerales (incluyendo calcio) o productos dietéticos especiales (salvado, aceite de onagra, leche con ácidos grasos omega-3, flavonoides, etc.), por favor indique la marca y la frecuencia con que los tomó:

Marcas de los suplementos de vitaminas o minerales o de los productos dietéticos	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6 +
140.									
140.1									
140.2									

140 (No marque aquí)

140.1 (No marque aquí)

140.2 (No marque aquí)

SUMCO 12637-03-(Ref. 2)

Muchas gracias por su colaboración

Annex 8

FULL D'INFORMACIÓ AL PACIENT

DADES DE L'ESTUDI

Títol de l'estudi: *Valoració del RAID-Dx com a test diagnòstic en pacients amb Malaltia Inflamatòria Intestinal en remissió i símptomes compatibles amb Intestí Irritable per a la diferenciació entre Síndrome d'Intestí Irritable envers una reactivació de la Malaltia Inflamatòria intestinal*
Investigador/a principal: _____
Centres: *Hospital Universitari Josep Trueta*, *Hospital Santa Caterina, Hospital de Figueres, Hospital d'Olot i comarcal de la Garrotxa, CAP de Montilivi i CAP Can Gibert del Pla.*

1. INTRODUCCIÓ

Ens dirigim a vostè per convidar-lo a participar, de manera completament voluntària, en un estudi que es realitzarà en persones que, com vostè, pateixen malaltia inflamatòria intestinal. L'estudi ha estat aprovat pel Comitè d'Ètica i Investigació Clínica (CEIC) de l'Hospital Universitari Josep Trueta de Girona, d'acord amb la legislació vigent, i amb respecte als principis enunciats en la declaració d'Hèlsinki i a les guies de bona pràctica clínica. La nostra intenció és que rebi la informació correcta i suficient perquè pugui avaluar i jutjar si vol o no participar-hi. Per això li preguem que llegeixi aquest full informatiu amb atenció i nosaltres li aclarirem els dubtes que li puguin sorgir. A més a més, podeu consultar-ho amb les persones que considereu oportú.

2. DESCRIPCIÓ GENERAL DE L'ESTUDI

¿Quina és la seva malaltia?

La malaltia inflamatòria crònica i la síndrome de l'intestí irritable són dues entitats cròniques i recidivants que es caracteritzen per produir clínica intestinal en forma de alteracions en l'hàbit deposicional (diarrea i/o restrenyiment) i dolor abdominal, entre d'altres. Si bé és cert que les dues entitats estan ben diferenciades, considerant-se la primera com una malaltia d'origen orgànic fruit de la inflamació de la mucosa intestinal i la segona com un trastorn funcional de la motilitat de l'intestí, comparteixen certes característiques, com les manifestacions clíniques, certs factors de risc, l'agrupació familiar i l'alteració de la microbiota intestinal, és a dir, el conjunt de poblacions bacterianes, fúngiques i víriques que coexisteixen amb nosaltres en el nostre intestí. A més a més, aquest la correlació entre ambdues entitats es fa encara més present quan es compara la prevalença de símptomes compatibles amb síndrome d'intestí irritable en persones que pateixen una malaltia inflamatòria intestinal en estat de remissió, com vostè, essent molt més alta (25% superior) respecte la població general. Un dels principals que existeixen en aquest context és la manca d'eines diagnòstiques òptimes per diferenciar entre una síndrome d'intestí irritable i una reactivació de la malaltia inflamatòria intestinal de base, excepte per la colonoscòpia. Així doncs, en pacients amb una malaltia inflamatòria intestinal de base en estat de remissió que acudeixen a la consulta per simptomatologia intestinal tipus

diarrees i dolor abdominal, la manera per diferenciar entre les dues entitats és a través de la realització d'una colonoscòpia, una prova invasiva i poc convenient per a qualsevol persona.

¿Perquè és necessari aquest estudi?

La malaltia inflamatòria intestinal és una entitat crònica i recidivant cada cop més prevalent entre la població general, especialment entre la segona i tercera dècades de vida. La causa de la malaltia és, encara, desconeguda. Tanmateix, és cert que se'n coneixen factors de riscs i d'altres relacionats amb la seva patogènia, com la composició de la microbiota intestinal. La proporció de les diferents espècies de microorganismes que coexisteixen en nosaltres crea un segell microbiològic, com una signatura. Aquest fet s'ha utilitzat per a la identificació de certes malalties com la malaltia inflamatòria intestinal o la síndrome de l'intestí irritable, fet que ha permès establir signatures microbiològiques definitòries per a cada una d'elles. Arrel d'aquests estudis, la microbiota intestinal està acumulant un gran interès tant com a eina diagnòstica com terapèutica.

L'objectiu de l'estudi en què se'l convida a participar és valorar l'efectivitat d'una nova eina diagnòstica, RAID-Dx, basada en la detecció de l'alteració de certes poblacions de la microbiota intestinal a partir d'una mostra de femta. D'aquesta manera, es podria aconseguir seleccionar de manera adequada aquelles persones que necessiten realment una colonoscòpia i evitar-la quan fos innecessària. Per aquest motiu, és convidat/da a participar en aquest estudi.

¿Quants centres hi participen i quant de temps dura?

Aquest estudi comptarà amb la participació de 6 centres de la regió sanitària de Girona, Figueres i Olot. S'inclouran un total de 207 participants que es reclutaran en el transcurs de 7 mesos aproximadament i l'estudi tindrà una durada estimada de 2 anys, en els quals no se li demanarà realitzar cap seguiment addicional ni exploració complementària.

¿Quines característiques han de reunir els/les pacients per participar en l'estudi?

Els/les participants han de tenir, com vostè, un diagnòstic clar de malaltia inflamatòria intestinal en estat de remissió, és a dir, sense haver requerit una intensificació o modificació del seu tractament de base en els últims 6 mesos, i experimentar símptomes compatibles amb un diagnòstic de síndrome d'intestí irritable. A més a més, es requereix tenir programada una colonoscòpia durant els 2 mesos següents a la manifestació dels símptomes.

¿En què consisteix la meva participació en l'estudi?

La seva participació en l'estudi consistirà en l'acceptació a unir-se a aquest, així com la recollida d'una mostra de femta, que es podrà realitzar durant la mateixa consulta o un cop vostè es trobi a casa. És important recol·lectar la mostra de femta en les condicions més estèrils possibles. Per tal d'aconseguir-ho se li proporcionarà un pot estèril que haurà de mantenir tancat fins que vagi a dipositar-hi la mostra. Un cop obtinguda, constarà d'un termini d'1 hora per tal de portar-la al centre de referència que haurà d'enviar-la fins als nostres laboratoris on s'haurà de congelar i conservar a -20°C. El transport fins al centre es pot efectuar a temperatura ambient. La mostra es guardarà al Biobanc fins al seu processament i, posteriorment, vostè té el dret de decidir com es gestionarà.

A part de la mostra de femta es requerirà, també, que ompli un formulari en relació als seus hàbits dietètics per tal de valorar possibles factors d'interacció amb la prova a estudi. A més a més, un treballador de la salut format li prendrà les dades bàsiques de pes i talla.

¿A què em comprometo si decideixo participar en l'estudi?

La participació en aquest estudi no comporta la realització de cap prova, exploració o visita complementària a la que vostè acaba de realitzar. Un cop realitzada l'obtenció de la mostra no se li demanarà de realitzar cap activitat addicional i, en el suposat cas de canviar el plantejament inicial de l'estudi, se li informaria abans de procedir a la utilització de les seves dades.

¿Tinc la possibilitat de consultar amb altres professionals?

Sempre que ho desitgi pot consultar amb altres professionals abans d'accedir a participar a l'estudi per demanar-los una segona opinió.

3. CONSENTIMENT

Si vostè està d'acord en donar la mostra femta, i la realització de les altres exploracions detallades en l'apartat anterior, cal que signi aquest formulari de consentiment. Després que vostè signi aquest formulari es recollirà la mostra de femta.

En el cas que es produís un desenvolupament comercial dels coneixements generats, els possibles beneficis que es poguessin rebre seran íntegrament destinats a satisfer els objectius científics del grup de recerca al seu manteniment. Signant aquest formulari de consentiment, vostè renuncia als seus drets sobre qualsevol ús comercial relacionat amb la informació o les mostres que vostè ha cedit.

Excepcionalment, i sempre que vostè així ho autoritzi, es podria tornar a contactar amb vostè per a sol·licitar informació addicional.

4. ÚS DE LES SEVES MOSTRES BIOLÒGIQUES

Les seves mostres biològiques i la seva informació personal recollida com a part del projecte "*Diferenciació entre Síndrome d'Intestí Irritable i reactivació de Malaltia Inflamatòria Intestinal a través del RAID-Dx en pacients amb Malaltia Inflamatòria Intestinal en remissió i símptomes compatibles amb Intestí Irritable"* seran utilitzades únicament per a la realització del mateix.

L'emmagatzematge de totes les mostres està previst realitzar-lo a les instal·lacions del Biobanc IDIBGI (B. 0000872). Una vegada finalitzat l'estudi, si vostè ho autoritza, l'excedent de les mostres que no hagi estat utilitzat passarà a formar part del Biobanc per poder ser utilitzat en estudis posteriors. És per això que se li facilita un segon full d'informació i consentiment específic que haurà de signar i que serà custodiat pel coordinador del Biobanc del seu Hospital.

El tractament i ús de les mostres es realitzarà seguint el que especifica la Llei de Recerca Biomèdica (14/2007), i el RD 1716/2011.

5. BENEFICIS I RISCS DERIVATS DE LA PARTICIPACIÓ EN L'ESTUDI

¿Quins riscs assumeixo si participo en l'estudi?

El test que es vol estudiar (RAID-Dx) es basa en l'obtenció de DNA bacterià a partir d'una mostra de femta, per la qual cosa la realització d'aquest no suposa cap risc a la seva seguretat personal.

L'extracció de la mostra no suposarà cap cost econòmic per vostè.

¿Quins beneficis obtindré de la meva participació a l'estudi?

La seva participació contribuirà a un millor coneixement de la seva malaltia i a l'estudi d'una possible nova eina diagnòstica, que podrà proporcionar futurs beneficis a les persones que la pateixin. També ha de conèixer que és possible que no obtingui cap benefici per la seva salut per participar en aquest estudi.

6. PARTICIPACIÓ VOLUNTÀRIA

Ha de saber que la seva participació en aquest estudi és voluntària i que pot decidir no participar-hi o canviar la seva decisió i retirar el consentiment i abandonar l'estudi en qualsevol moment, sense donar cap tipus d'explicació. Si decideix retirar el consentiment per participar en aquest estudi, no s'afegirà cap dada nova a la base de dades i pot exigir la destrucció de totes les proves identificables prèviament retingudes per evitar la realització de noves anàlisis, si bé els responsables de l'estudi poden continuar utilitzant la informació recollida sobre vostè fins aquell moment, tret que s'hi oposi expressament. Per suposat, encara que vostè abandoni l'estudi seguirà rebent la mateixa atenció sanitària per part dels seus metges.

7. INFORMACIÓ DELS RESULTATS DE LA INVESTIGACIÓ

Els estudis que es puguin incloure en el pla de recerca del projecte tenen un objectiu epidemiològic fonamentalment i no consten d'utilitat clínica immediata. És més, en funció del pla de recerca i la coordinació de l'equip, pot passar un lapse de temps considerable des de l'extracció fins a la realització de l'estudi. És a dir, els temps de la investigació dependrà de la inquietud i recerca que es vagi desenvolupant en la comunitat científica i poden no correspondre's amb els temps de l'atenció clínica, generalment més curt. Els mètodes utilitzats en investigació biomèdica solen ser diferents dels aprovats per a la pràctica clínica, per la qual cosa no han de ser considerats amb valor clínic per a l'individu. No obstant això, en el cas que aquestes investigacions proporcionessin dades clínica o genèticament rellevants per a vostè i interessar la seva salut o la de la seva família, li serien comunicades si així ho estima oportú. De la mateixa manera, podria donar-se el cas d'obtenir informació rellevant per a la seva família; si es produeix aquesta situació, li correspondrà a vostè decidir si vol o no comunicar. Si vostè vol que se li comuniqui aquesta informació rellevant, ha de marcar la casella corresponent al final d'aquest document. Si vostè no desitja rebre aquesta informació, tingui en compte que la llei estableix que, quan la informació obtinguda sigui necessària per evitar un greu perjudici per a la salut dels seus familiars biològics, un comitè d'experts estudiarà el cas i haurà de decidir si és convenient informar als afectats o als seus representants legals. Si per qualsevol motiu vostè

volgués conèixer els resultats de les investigacions que s'han produït com a conseqüència de la seva col·laboració, podrà posar-se en contacte amb els responsables del projecte, que l'informaran degudament sobre els resultats d'aquest.

8. PRIVACITAT I CONFIDENCIALITAT

Per la realització de l'estudi es recolliran dades mèdiques sobre la seva persona i es generarà informació sensible sobre vostè. La recollida i anàlisi posterior de totes aquestes dades es realitzarà garantint estrictament la seva confidencialitat d'acord amb l'establert en el "Reglament (UE) 2016/679 del Parlament i del Consell, de 27 d'abril de 2016, relatiu a la protecció de les persones físiques pel que fa al tractament de dades personals i a la lliure circulació d'aquestes dades i pel qual es deroga la Directiva 95/46/CE (Reglament general de protecció de dades) (DOUE 4.5.2016)". La seva privacitat està protegida per ambdues lleis, les quals busquen evitar accessos involuntaris a la informació generada que podria comprometre l'exposició dels participants a l'estudia, i a les seves famílies, a efectes adversos tant econòmics, legals, psicològics i/o socials. A més a mes, només aquelles dades que estiguin relacionades amb l'estudi seran objecte de registre, en cap moment s'utilitzaran el seu nom, adreça o qualsevol altra informació que pugui identificar-lo. A tot això, cal afegir que d'acord a la LO15 / 1999, vostè pot exercir els drets d'accés, rectificació, oposició i cancel·lació de les dades; per això, haurà de contactar amb l'Investigador Principal del projecte.

Per protegir la seva privacitat, les seves mostres de femta tindran un codi alfanumèric perquè no es puguin identificar a partir del nom. Vostè té el dret de sol·licitar la destrucció de les mostres en qualsevol moment de l'estudi. A part de la mostra en sí, les seves dades personals requerides per a registrar-lo com a participant de l'estudi seran identificades mitjançant un codi per tal d'assegurar i mantenir la confidencialitat de les mateixes. Només els investigadors responsables del projecte coneixeran la identitat rere cada mostra, informació necessària per a la realització de l'estudi. Així doncs, l'accés a la seva informació personal quedarà restringit al/la metge/metgessa de l'estudi, col·laboradors, autoritats sanitàries, al Comitè d'Ètica i personal autoritzat, quan ho necessitin per comprovar les dades i els procediments de l'estudi, però sempre mantenint-ne la confidencialitat d'acord amb la legislació vigent.

9. DUBTES I AGRAÏMENTS

¿Amb qui he de contactar davant qualsevol dubte o problema que sorgeixi?

En cas de necessitar informació o comunicar qualsevol esdeveniment que succeeixi durant la realització de l'estudi, podrà posar-se en contacte amb el Dr/a. : _____, a través del número de telèfon _____ o correu electrònic _____.

Sigui quina sigui la vostra decisió, l'equip investigador vol agrair-vos el vostre temps i la vostra atenció.

Signatura del/a pacient	Signatura de l'investigador/a
Nom:	Nom:
Data:	Data:

FULL DE CONSENTIMENT INFORMAT DEL/LA PACIENT

Jo,_____ (nom i cognoms), declaro:

- Haver llegit i entès el full d'informació que se m'ha lliurat.
- Haver pogut fer les preguntes que m'hagin sorgit sobre l'assaig i haver-les resolt.
- Haver rebut informació suficient sobre l'estudi.
- Haver entès el meu paper com a participant en l'estudi

Declaro, també, haver entès que la meva participació és voluntària i puc retirar-me de l'assaig:

- Quan vulgui.
- Sense haver de donar explicacions.
- Sense que això repercuteixi en les meves cures mèdiques

De conformitat amb el que estableix el Reglament (UE) 2016/679 del Parlament i del Consell, de 27 d'abril de 2016, relatiu a la protecció de les persones físiques pel que fa al tractament de dades personals i a la lliure circulació d'aquestes dades i pel qual es deroga la Directiva 95/46/CE (Reglament general de protecció de dades) (DOUE 4.5.2016), declaro haver estat informat de:

- L'existència d'una base de dades on s'inclouran les meves dades de caràcter personal.
- De la finalitat de la seva recollida i dels destinataris de la informació.
- Del procés de codificació de les dades.
- De la disponibilitat d'exercir els drets d'accés rectificació, cancel·lació i oposició dirigint-me per escrit al titular de la base de dades.

En relació a les mostres proporcionades, entenc:

- Els meus drets com a pacient de la investigació, i voluntàriament accedeixo a cedir el meu material biològic i genètic.
- Poder sol·licitar la retirada i/o eliminació de les mostres proporcionades en qualsevol moment i sense donar explicacions.
- El propòsit del projecte i com el meu material biològic i genètic seran utilitzats.
- Els meus drets com a pacient de la investigació, i voluntàriament cedeixo el meu material biològic i genètic.

Desitjo ser informat dels resultats clínicament rellevants

Sí ☐ No ☐

Accedeixo a què es em contacti en un futur en el cas que es consideri oportú afegir noves dades als recollits inicialment, o mostres biològiques addicionals

Sí ☐ No ☐

DESTINACIÓ MOSTRA DESPRÉS DE LA SEVA UTILITZACIÓ EN AQUEST PROJECTE DE RECERCA

Un cop finalitzada la investigació, és possible que hi hagi mostres sobrants. En relació a aquestes, se li ofereixen les següents opcions:

A. Destrucció de la mostra sobrant.
B. La introducció de l'excedent de la mostra a un Biobanc
   En aquest cas, se li facilitarà full d'informació al pacient i consentiment específic que haurà de signar i serà custodiat pel coordinador del Biobanc del seu Hospital.

Si hagués excedent de la mostra, afirmo haver estat advertit sobre les opcions de destinació en finalitzar el projecte de recerca. En aquest sentit:

- Sol·licito la destrucció de la mostra excedent ☐
- Permeto que les meves mostres siguin introduïdes en el Biobanc de l'hospital ☐

LA SEVA SIGNATURA INDICA QUE HA LLEGIT I ENTÉN LA INFORMACIÓ ESMENTADA EN AQUEST DOCUMENT, QUE HA DISCUTIT AQUEST ESTUDI AMB LA PERSONA QUE OBTÉ AQUEST CONSENTIMENT, QUE HA REBUT UNA CÒPIA D'AQUEST FORMULARI I QUE DÓNA LLIUREMENT LA SEVA CONFORMITAT PER PARTICIPAR EN AQUEST ESTUDI.

Data:_____ Signatura: _____