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TESIS DOCTORAL

CLOSTRIDIUM DIFFICILE INFECTION IN INFLAMMATORY BOWEL DISEASE: RISK FACTORS, RECURRENCE AND OUTCOME

Tesis Doctoral presentada por Dña. Mónica Peñate Bolaños

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Ph. D. DISSERTATION

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MÓNICA PEÑATE BOLAÑOS

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Acknowledgments

To my promotors:

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To Julia Foster

To my colleagues

To Dr. Esteban Perez Alonso

And finally, to my patients for teaching me every day to be a better person

I have a dream….

Martin Luther King, 1963

Foreword

How long has this thesis been going? Why right now? I would like to share with you, my professional and personal reasons that have brought me here.

My professional way started in 2010. We began to study for the first time the impact of Clostridium difficile in patients in our centre in collaboration with the Microbiology Department. The results of this study were presented as an oral communication to both, the Regional Congress of Canarian Society of Digestive Pathology and National Congress of Internal Medicine in 2011. When we analysed the results, we found Clostridium difficile infection was more frequent in hospitalized patients except in inflammatory bowel disease group. Moreover, when we analyzed their characteristics in a separate way, we got completely different results compared with those from the rest of patients. For that reason, we planned to study Clostridium difficile infection in more detail. Therefore, we started a first descriptive study in out and inpatients in 2013. It was presented as oral communication in the Regional Congress of Canarian Society, and we won the prize for the best oral communication. After this, I decided to perform this thesis adding a control group.

My personal journey started 13 years ago. I was in Amsterdam in my external rotation at the end of my training in the Digestive specialization with Dr. Salvador Peña. At that moment, I was pregnant with my first child. Thus, what happened next? Being alive involves making decisions all the time and I chose my family at that moment. However, I did not give up my dream, and here I am! It has been a long journey, and I have had an excellent good travel companions: Elena Jiménez and Alberto Monescillo who have always been my stronger supports. They are my colleagues, my friends, the shoulder to lean on when everything collapses, and it happened more than once. During all this time, I have been interested in different subjects such as psychological disorders in IBD patients. The years have gone by very fast but my necessity to carry out a thesis continued and finally in 2013 this project began. First of all, I needed to refresh my rusty English, so I returned to study English and to certificate the B1 and B2 levels with Cambridge and Trinity College respectively.

It has been a very long process but completely rewarding. Now, it is the second moment most important in my professional life. I would like to share it with all people who love me and I love.

Anywhere but here, anywhen but now

Terry Pratchet

To Dad and Mum:

For my education, a world full of possibilities

To Toni and Luna:

You are my life

To Jose:

Thank you for giving me time, the most valuable present

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Abbreviations

IBD: Inflammatory bowel disease

C. difficile: Clostridium difficile

C. diff: Clostridium difficile

CDI: Clostridium difficile infection

CD: Clostridium difficile

CD: Crohn´s disease

UC: Ulcerative colitis

FMT: Faecal microbiota transplant

IM: Immunomodulators

AB: Antibiotic

General introduction

1. General introduction

Clostridium difficile infection (CDI) in inflammatory bowel disease (IBD) patients is a fascinating subject. Clostridium difficile (CD) is a cause of diarrhoea in humans for more than 30 years, and its incidence has been increasing in the last decade. Since the early 2000s, severe CDI outbreaks in hospitalized patients in North America and Europe have put in check to the health authorities, and it has become a serious health problem with a high rate of complications and mortality. Newspapers and television have covered the major Clostridium difficile outbreaks in the UK and North America. Recently, in September 2015, the first faecal bank for recurrent CDI treatment has been created in the USA. It provides clear evidence that the number of people contracting this hard-to-control and treat bacterial infection is increasing.

What is Clostridium difficile and why is so hard to control and treat?

CD is the most frequent cause of diarrhoea in hospitalized patients and antibiotic-associated colitis. It is a Gram-positive bacteria, strict anaerobic and spore-forming. Spores are easily spread via airborne and can persist under adverse conditions for a long time. It can contaminate hospital environment by spread through health care workers and suboptimal environmental cleaning practices.

CD lives harmlessly in the gut of 10% to 15% of adults under normal conditions without causing symptoms (colonization). However, when the balance of intestinal flora is disrupted, often following a course of antibiotics, it may produce a wide spectrum of clinical manifestations ranging from asymptomatic carriage and uncomplicated diarrhoea to a severe, life-threatening infection.

In the last decade, the severity and mortality have increased, due to more virulent strains (e.g. B1/NAP1/027) resistant to conventional antibiotics treatments. Thus, we have had many problems with the treatment and the prevention onset new infections and recurrences. Treatment includes infection control measures. Antibiotic therapy should be initiated as soon as possible, and faecal microbiota transplantation can constitute another alternative treatment for recurrent and severe Clostridium difficile infection (CDI).

Which patients are at risk to be affected by CD?

It had been believed that the typical profile of a patient with CD was a hospitalized and elderly patient, undertaking antibiotics, with chronic diseases and immunodeficiency. However, the number of people who contract it is increasing out of hospitals. The antibiotics exposure is neither necessary nor

sufficient for it, for the first time, an increased incidence of CD in children and pregnant women has been described. A group particularly susceptible to CDI is inflammatory bowel disease (IBD) patients (Crohn's disease and ulcerative colitis) especially those with involvement of the colon. IBD patients with CDI tend to be younger, have less prior antibiotic exposure and in most cases had a community acquisition. Consequently, IBD patients have a different risk profile compared with general population.

Which is the relationship between Clostridium difficile and inflammatory bowel disease? May CDI be as dangerous as in the general population?

The relationship between CD and IBD is controversial. It is still not clear whether CD is an etiologic cause or a consequence. It is unknown whether the problem is caused by an alteration of the local immune system in the intestine or the systemic inflammatory state or both. CDI can play an important role in the clinical initiation of IBD, can produce a delay at IBD diagnosis and can be a challenging factor in the differential diagnosis of relapses.

CDI adds difficulties in IBD therapeutic management because it needs a specific antibiotic treatment. Several studies in IBD have demonstrated a rising in the severity and rates of recurrence, both associated with an increase in morbidity, surgeries and mortality.

Could the recurrence of CDI be a problem in IBD patients? Which would the best therapeutic approach be?

The recurrences may be a problem after a first treatment in non-IBD and IBD patients, around 30% in both.

In IBD, the investigation of CD in stools sample is recommended in the relapses resistant to conventional treatment in outpatient and all inpatients, based on clinical practice European, American and Spanish Guidelines. However, there are not specific recommendations for the treatment in this patients. Nowadays, we treat our IBD patients based on Microbiology Guidelines. For this reason, it would be important that we had specific protocols for the best treatment strategies in IBD patients specifically.

We have performed a retrospective, case-control study to evaluate the following points in our country:

How have the *number of IBD patients* with CDI changed in the last 8 years?

Which are the *risk factors* for CDI in IBD patients with a relapse in our country?

Is the *recurrence* frequent in our patients? How do we *treat* them?

How do we *treat* CDI in our IBD patients?

How does CDI affect the *outcome of IBD?*

Our study is the first made in the Canary Islands about CDI in IBD patients to add experience in the risk factors, recurrence and outcome. We need to know the implications of this infection in IBD patients with a relapse in our country.

To attempt to answer those questions, the ***outline*** of this thesis is:

Chapter 1: Theoretical framework, includes an update of most important aspects that contribute to understanding this study and its results. Special attention is given to the influence of Clostridium difficile on inflammatory bowel disease.

Chapter 2: Study: Aims and method section

Chapter 3: Results

Chapter 4: Discussion

Chapter 5: Conclusions

Chapter 6: Our proposals to modify the management of CDI in IBD patients in our area, according to our study

Appendices

Bibliography

Summary in Spanish

Chapter 1

2. Chapter 1: Theoretical framework: Clostridium difficile and IBD

2.1 Introduction

IBD is a chronic intestinal inflammation whose etiology and pathogenesis are not yet fully known. On the whole, Crohn´s disease (CD) and ulcerative colitis (UC), are polygenic and multifactorial diseases. In general, it has been suggested that several environmental, microbial, immunologic, genetic and lifestyle factors play a role in their initiation. In studies from the 1980s to present, Clostridium difficile (CD) has been implicated to be a risk factor for relapses of the inflammatory process in up to 5% of patients with IBD. Several studies in the last 10 years have reported higher rates of CD colonization (CDC) and CDI in patients with IBD. However, CD toxin has been detected in patients with inflammatory bowel disease, especially with symptomatic relapses. In some episodes, no prior antibiotic administration was recorded, and symptoms responded to vancomycin. Previously, some "relapses" have been produced for "disease activity" of the underlying inflammatory bowel disease. Some physicians thought that some medical treatments (e.g. sulfasalazine) could alter the intestinal flora and promote CD colonization. Others theorized that altered immune status, possibly related to therapeutic agents, or nutritional status could be important. Thus, IBD patients are considered a risk group for CDI but the risk of infection cannot be fully explained by the well-known risk factors, in the general population and it makes the question whether abnormalities in mucosal immune response in IBD could play a role in CDI.

In the late 1970s, the investigation of stools samples for the presence of Clostridium difficile toxins in relapses of IBD was not recommended. In 2002, in the Digestive Disease Week meeting in the USA, the presence of C. difficile and its toxins were described for the first time in a significant number of patients with inflammatory bowel disease (IBD). The significance of this association was still not known, but it said that accurate tests could help ensure that these patients received the appropriate treatment. In these last years, the latest European, American and Spanish guidelines recommend investigating CD in IBD relapses.(1) So, *what has changed? Is necessary the routine investigation in all IBD patients with a relapse or only in some specific situations?*

In this chapter, our goal is to provide an overview of current knowledge on Clostridium difficile and the relationship between Clostridium difficile and inflammatory bowel disease. Our purpose is to understand the role that CD plays in relapses in IBD patients. We focus on the relevant information useful to follow our line of argument in the elaboration and interpretation of the results of our study.

First of all, we are going to explain how we did the research for the information to elaborate the theoretical framework. Secondly, this review is going to summarize the most important aspects of CDI and its relationship with IBD and finally, we are going to review how we can diagnose and treat CDI.

Update methodology

We searched on the PubMed in English language, medical literature from 2000 to September 2015 using the terms (search terms) (search strategy):

‘ Clostridium difficile AND outbreak’ ‘ Clostridium difficile AND inflammatory bowel disease.'

‘ Clostridium difficile AND inflammatory bowel disease AND risk factors.'

‘ Clostridium difficile AND inflammatory bowel disease AND treatment.'

‘ Clostridium difficile AND inflammatory bowel disease AND outcome.'

All randomized and non-randomized trials, cross-sectional, cohort and case-controlled studies published in English were included. The resulting literature (abstract and most relevant full texts) was reviewed. Furthermore, meta-analysis and the updated guidelines for treatment and diagnosis of Clostridium difficile infection were evaluated.

2.2 What is Clostridium difficile?

The human intestine contains trillions of bacteria, the major phyla of which include Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria. Clostridium difficile (CD) is a gram-positive, anaerobic, the rod-shaped bacillus that produces spores and spreads via faecal-oral route. It is in the bowel of 4-13% of asymptomatic people. (2) The organism was first identified by Hall and O’ Toole in 1935 in the stools of newborns and referred to as Bacillus Difficilis due to the difficulty in isolation and study. The organism was renamed Clostridium difficile.

CD is a pathogen of both, humans and domestic animals. (3) Clostridium difficile-associated disease is of real importance in humans and has been a not-uncommon cause of enteric disease in horses, dogs, and pigs (4). Given the widespread occurrence of the disease, there is a substantial effort to develop immunoprophylactic products (5). CD is an emerging pathogen in animals, suggesting that food could be involved in the transmission of CD from animals to humans. (6, 7) Recently, Mooyottu et al. (8) detected a genotypically similar and identical CD strains implicated in human infections from food animals (ground beef, pork, and chicken) indicating the potential role of food as a source of community-associated CD.

Also, Kotila et al.(9) demonstrated for the first time the contamination of a tap water distribution system and water transmission of CD and Steyer et al.(10) described the occurrence of Clostridium difficile in the effluent of a wastewater treatment plant (WWTP) during a 1-year period. Pathogens detected in WWTP effluent reflected the epidemiological situation of enteric viruses and bacterias in the human population and the importance of the treatment of these pathogens before release into the surface water system.

It has been demonstrated that environmental surfaces in the rooms of patients with CDI can be contaminated with spores. However, we have less information about the contamination of environmental surfaces outside of CDI isolation rooms. Dumford et al. (11) performed a study to investigate the presence of C difficile in rooms of patients not in isolation for CDI, in physicians and nurses work areas, and on portable equipment. They found that environmental contamination was common in non-isolation rooms, in physician and nurse work areas and on portable equipment. We do not the real value of this contamination, related to CD transmission.

The horizontal transmission of CD in the hospital environment is difficult to demonstrate. Current methods to detect C. difficile spores on surfaces are not quantitative and have low sensitivity. Ali et al. (12) proposed a new rapid method to detect and quantify C. difficile contamination on surfaces: sponge swabbing. They found that this technique could be used for routine cleaning surface and as a tool to investigate routes of patient-patient transmission in the clinical environment.

Xu et al. (13) study the sanitary status and incidence of methicillin-resistant Staphylococcus aureus and Clostridium difficile within Canadian hotel rooms. The authors' study demonstrated that hotel rooms represent a potential source of community-acquired infections and the need for enhanced sanitation practices.

In another study, a hospital outbreak of CDI was linked to a laundry machine malfunction so CD spores could survive on surfaces for prolonged periods of time. These spores can contaminated the hospital environment by spreading through health care workers and suboptimal environmental cleaning practices. (14)

CDI is a recognized cause of infectious hospital-acquired diarrhoea in the developed world. Moreover, it is the main cause of antibiotic-associated disease, a disease of high importance socio-economic. However, there is an increasing recognition of CD in children, healthy adults and pregnant women. CD was reported for first time in 1978, as the major cause of antibiotic-associated pseudomembranous colitis. (15) Over the last 15 years, there has had a marked increase in the incidence of CDI, leading to increase a research interest at the discovery of new virulence factors and in the development of new treatment and prevention regimens. The new strain of C. Difficile, characterized as toxigenic type III, CRP ribotype 027 (C. difficile 027), presents higher pathogenicity because of

increased exotoxin production, and its antibiotic resistance profile. It has been involved in recent hospital outbreaks and community-acquired infections. Since 2003, several European countries and Canada have notified cases of C. difficile 027-associated disease. This fact demonstrates its rapid dissemination and generate concern among health and no health professionals.

After 1977, several studies discovered two potent toxins produced by CD and rather than the organism, were responsible for significant and sometimes severe inflammatory changes in the colon. Only toxigenic strains with a pathogenicity locus (PaLoc) cause disease: enterotoxin A (TcdA) and cytotoxin B (TcdB). (16) In addition to the two toxins (TcdA and TcdB), some strains of Clostridium difficile also produce an actin-specific ADP-ribosyltransferase, called binary toxin (CDT) (4%). (17) Enterotoxins A and cytotoxin B play a major role in its pathogenesis, and the detection of these toxins in gut content has been the gold standard for diagnosis for many years. However, CRP ribotype 017, one of five clonal lineages of human-virulent C. difficile, lacks TcdA expression but causes widespread disease. (18-20) In general, the factors that mediate the disease include the dose and toxigenic of the colonizing strain, its ability to adhere to the colonic epithelium (infects the internal lining of the colon), the presence of others organisms that affect its multiplication and toxin production and the susceptibility of the host. This produces a disruption of the tight junctions, inflammation and damage to the intestinal mucosa, with the characteristic “volcanic eruption” observed in pseudomembranous colitis.

Hypervirulent strains, such as ribotype 027, has been described worldwide. It expresses the binary toxin, which damages human cells by inhibiting actin polymerization (21) and encodes a TcdC mutation that results in a truncated, inactive TcdC protein. This results in unsuppressed and unregulated toxin production, and levels of toxins A and B are 16 and 23 times higher in patients with this strain (hyperproduction of toxins A and B). (22) The dissemination of this strain in North America and Europe could change the epidemiology of C difficile-associated disease. (23) In 2003, in Quebec (Canada), a strain of CD resistant to fluoroquinolones, with the binary toxin and an incomplete deletion of the tcdC gene caused an outbreak. (24) McEllistrem et al. (25) found that the severity of CDI was not associated with a particular clone or underlying disease, but it could associate with the presence of the binary toxin genes. In this study, the binary toxin genes were detected in approximately 5% of CD strains.

2.3 Relationship between Clostridium difficile and inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic, immune-mediated disease of the gastrointestinal tract that develops in genetically susceptible individuals. However, in the pathogenesis of IBD, the alteration in the intestinal microflora may disrupt its homeostasis. Dysbiosis in IBD predisposes to colonization with C. difficile. There is a higher proportion of asymptomatic carriers in IBD than in the general population. (26) In the past, it was not thought that CDI in IBD patients was relevant. In fact,

Rolny et al. in 1983 did not recommend to investigate CDI in IBD patients with a relapse. The frequency of CDI in IBD patients has doubled or tripled since 2001. We do not know whether CD is a cause of IBD or a consequence of the inflammatory state in the intestinal environment and disruption of the normal microbiota.

Although it had not been possible to demonstrate that Clostridium difficile played an etiologic role in the IBD pathogenesis, it was demonstrated it played a role in relapses. (27) CD can promote relapsing of CU by activating the immune response. CD toxins may mediate mucosal inflammation, together with cell wall components of the microorganism. Innate and adaptive host responses to CD toxins and the role of mucosal changes in IBD may increase the inflammatory response in the presence of CDI. Thus, the restoration of intestinal flora and colonization resistance is thought to be the mechanism responsible for the treatment of recurrent CDI (28) and on the other hand, infliximab treatment can be useful and protective due to this activation of the immune response. (29)

IBD is one of the strongest comorbidities associated with the possibility of CDI. (30) Patients with IBD have a higher incidence of CD in comparison with the general population. (31, 32) CDI is important in the clinical initiation of IBD. The prevalence of CDI in newly diagnosed IBD patients is high (8.1-10%) and is independent of the type of disease. The risk of CDI in new onset paediatric IBD was associated with an increase in the age of the patient and the severity of the disease (33, 34) but the specific risk factors reported in adults were not identified in children, suggesting the possible involvement of other mechanisms for acquiring the pathogen. (35)

However, questions about the role of infections in the development and exacerbations of inflammatory bowel disease remain unanswered. Last years have increased the research of the role of intestinal microflora in the pathogenesis of IBD. (18) Gut microbiota plays a role in the initiation of CDI in IBD patients by producing superinfection but also as causative agent. (36) The normal gut flora acts as a colonization barrier that protects against CD and this function may be compromised when gut flora is disturbed. The risk of CDI affects the colon, increases with use of cephalosporin and the presence of gastrointestinal feeding devices. These are important risk factors for community-associated CDI in children. (37) Hourigan et al. (38) studied the changes in the microbiota in children with IBD and CDI compared with children with CDI without IBD. They found that children with IBD presented more alterations of their microbiota compared with non-IBD children. Moreover, after CDI treatment with faecal transplantation (FMT) in children without IBD the microbiota restoration was complete and in those with IBD, bacterial diversity returned to pre-FMT baseline by 6 months, suggesting IBD host-related mechanisms modify faecal microbiome diversity.

2.4 Contrasting Clostridium difficile infection in IBD patients and general population

2.4.1 Overview

Over the past two decades, there has had an increase worldwide in incidence and severity of CDI in the general population and paralleling in IBD. The emergence of a hypervirulent strain in the early 2000s associated with an increase in the number and severity of CDI episodes in the US, Canada, and other countries has changed the management of CDI. The appearance of the NAP1/BI/O27 strain in the early to mid-2000s has been associated with more severe forms of CDI. Outbreaks have not been described in IBD patients until now.

2.4.2 Clinical presentation

CDI is a cause of antibiotic-associated diarrhoea. The prevalence of CDI in IBD patients has increased over last decades. CDI incidence in IBD has increased and it is higher than in non-IBD population. The increase in the number of cases may reflect an increase in the rising incidence of CDI in general or increasing the virulence of the organism as we have just commented previously. Clostridium difficile (CD), specifically its toxins, have been implicated as a risk factor for exacerbation of the inflammatory process in up to 5% of patients with ulcerative colitis or Crohn´s disease. (39)

Clinically, CDI may range from an asymptomatic carrier state to serious life-threatening colitis. The symptoms may develop within 48 to 72 hours after infection or may be delayed for 2 to 3 months, usually after the administration of antibiotics (in some cases, only a single antibiotic tablet may lead to severe disease). Clinical symptoms of CDI and an exacerbation of inflammatory bowel disease are clinically indistinguishable.(40) Therefore, an early suspicion is essential to start antibiotic treatment with/without corticosteroids.(41)

On the other hand, endoscopy rarely shows pseudomembranes and it is useless for diagnosing CDI in IBD.(42) Pseudomembranes and fibrinopurulent eruptions are not seen endoscopically or histologically.(43, 44). Ben-Horin et al. (45) evaluated the rate of pseudomembranes in IBD patients, identifying predictive factors for pseudomembranes' presence and assessing its clinical impact. This study documented that hospitalized IBD patients with CDI had low rates of endoscopic pseudomembranes, which were not produced by the use of immunosuppressant drugs. IBD patients with CDI and pseudomembranes presented more commonly fever, but their clinical outcome was similar to patients without pseudomembranes.

2.4.3 Risk factors

IBD patients

The traditional risk factors for CDI, in the general population include: hospitalizations, antibiotic use, older age and severe comorbidities. Moreover, infection with ribotype 027 predicts severe CDI and higher mortality and the use of antibiotics is a modifiable risk factor for severe CDI.(46) Contrasting, IBD patients have different characteristics: younger age, community acquisition, lack of antibiotic exposure, colonic IBD, and corticosteroids use. CDI can occur in the small bowel, in UC patients with an ileal pouch-anal anastomosis after a colectomy.(40)

Most studies have demonstrated that patients with inflammatory bowel disease (IBD) have a higher incidence of CD compared with the general population.(31) IBD and UC, in particular (colonic involvement), are the comorbidities most strongly associated with the possibility of CDI.(30, 47) In contrast, a study in German in 2011 found that a low percentage of hospitalized patients with relapses had CDI (48) and another performed in 2013 by Penders et al. (49) found that CD was not a common trigger for exacerbations of IBD in clinical in the Netherlands.

Most of IBD patients appear to contract CD as outpatients. CDI confirmed within 48 hours of admission, suggesting a community acquisition.(39, 42, 43) However, it can occur in hospitalized adults and children: among children, the rate of CDI was over 12 times higher in IBD than non-IBD hospitalizations and, among adults the rate of CDI was four times higher in IBD than non-IBD hospitalizations. In adults, CDI was significantly higher in ulcerative colitis (UC) than Crohn's disease but in children there was no differences between UC and CD (50). The incidence of CDI is 1.8-5.7% in hospitalized patients with ulcerative colitis (UC) (29). Sandberg et al.(51) found CDI-related hospitalizations were associated with longer lengths of stay than hospitalizations without CDI in children and young adults with IBD in the United States.

Colonization of the small bowel occurs more frequently in IBD patients with ileal-anal pouch anastomosis after colectomy. Small-bowel bacterial flora, such as the neoterminal ileum, is colonized by colonic type bacterial flora, which may make it susceptible to overgrowth with C. difficile, particularly with concomitant antibiotic treatment. Chronic or refractory pouchitis and cuffs can appear due to CD colonization. Surgeries involving only the left side of the colon with preservation of the ileocecal valve do not increase the risk of CDI of the small bowel. Other risk factors for CDI in IBD patients are: malnutrition, anaemia, HIV infection, dementia, immunosuppressed treatment, antibiotic exposure and proton pump inhibitors.(30, 43, 52)

Children with IBD, similar to adults, have an increased risk of acquiring a Clostridium difficile infection. CDI represents a significant healthcare burden in hospitalized children with IBD. (53-55). Paediatric patients with CD tend to have active colonic disease and a more severe disease course. (55, 56) Rate of recurrent CDI in children was 22%, independent of the type of IBD, and was significantly associated with: malignancy, recent surgery, and the number of antibiotic exposures. Wultanska et al. (57) and Bossuyt et al. (58) did not find significant correlation between CDI and IBD therapy. Other authors found immunosuppressant treatment as risk factors. (42, 59)

The latest risk factors:

In 2012, Shakir et al.(60) studied serum antibodies against C. difficile toxins for the first time. They were detected in susceptible populations and could be protective. However, these antibodies had not been studied in IBD patients. This study measured immunoglobulin G antibody levels to CD toxin B in serum from IBD patients in remission and IBD patients in relapse. IBD patients demonstrated significantly higher antibody levels than non-IBD patients. Also, more proportion of IBD patients in remission had positive antibody levels compared with IBD patients in relapse. The authors concluded that the characterization of antibody responses could improve our understanding of susceptibility to CDI among IBD patients.

Connelly et al.(61) identified a single-nucleotide polymorphisms (SNPs) associated with CDI among IBD patients. The interleukin-4 gene-associated SNP rs2243250 was strongly associated with CDI in IBD population. Thus, SNP could allow for the identification of IBD patients at greater risk for CDI.

Ananthakrishnan et al.(62) found that the therapeutic supplementation of vitamin D could be useful to prevent CDI. Plasma calcifediol [25(OH)D] stimulates the production of cathelicidins. Cathelicidins are anti-microbial peptides that attenuate colitis and inhibit the effect of clostridial toxins. The authors found that a higher plasma calcifediol [25(OH)D] was associated with reduced risk of CDI in patients with IBD.

Non-IBD patients

Most well-known risk factors for CDI in the general population are: antibiotic use (19, 37, 63-74), older age (>65) (19, 65, 73, 75-77), immunosuppressant treatment (19), immunocompromised patients (66, 78, 79), proton pump inhibitors (73, 80, 81), intensive care unit (75, 82), mechanical ventilation, prolonged hospital stay (65, 83), chemotherapy (79, 84), solid organ transplant (85), malignancies (79, 82), surgeries (antibiotic prophylaxis) (81), dialysis (86, 87), gastrointestinal surgery, nasogastric tube placement (19), contact with infected patients (88, 89), nutritional status (70, 76), diabetes (73, 90) In HIV infection, low gammaglobulin levels and low albumin levels at admission are associated with an increased risk of developing CDI. A deficiency in humoral immunity appears to play a major role in the development of CDI. (91) A traditional risk factor for CDI include hospitalization but a study performed in Brazil found 81% of CDI community acquired. (92)

Other risk factors described more recently are:

C. difficile is an important cause of healthcare-associated diarrhoea among paediatric patients. It was found that more than three-quarters of cases (75%) of CD were contracted in the community, not in the hospital. The incidence of CDI in children was 12 times higher between 2004 and 2009, compared with the period between 1991 and 1997 (32.6 cases per 100,000 vs. 2.6). (93) Fiedoruk et al. (94) determined CD were the main causative agent of community-acquired acute diarrhoea in children (14.8% of children aged >1 year).

Peripartum women appear to be another population susceptible to CDI. The use of a combination of antibiotics remained a significant independent risk factor. (95)

Van der Wilden et al.(96) found a significant inverse association between 25(OH) D3 levels and CDI severity in hospitalised adults. Further studies are needed to demonstrate whether vitamin D supplementation can improve outcomes in patients with CDI.

Perioperative antibacterial prophylaxis: 1.5% of patients who received perioperative antibacterial prophylaxis developed CDI. The independent risk factors associated with CDI were: older age, administration of Cefoxitin alone or in combination with another antibiotic and years of surgery. (97).

Clayton et al.(98) studied outbreak CD in a residential home in the UK. They found CDI affected residents had received a mean of 2.7 antibiotic courses in the two months preceding diagnosis. However, the investigation of the facility discovered problems with hand hygiene and environmental cleaning. The authors concluded that we need a health- and social-care systems working together to assure the

safety of people in their care. Zarowitz et al. (89) studied the incidence of CDI in nursing home residents and concluded that was high because of: decreased the immune response, multiple comorbidities, treatments, increased risk of infection, the proximity of residents, and recent hospitalization.

Patients with toxigenic CD colonization are at risk of developing CDI. Rates of asymptomatic CD colonization on hospital admission range between (1.4-21%). There are three main risk factors for C. difficile colonization: recent hospitalization within 3 months, chronic dialysis and corticosteroid use. (99) Lin et al.(100) found that risk factors for the development of CDI among hospitalized patients were: diabetes mellitus and recent piperacillin-tazobactam or PPIs treatments. Kong et al.(101) evaluated host and bacterial factors associated with colonization on admission. The hospitalization within the last 12 months, use of corticosteroids, prior CD infection, and the presence of antibody against toxin B were associated with colonization on admission.

Vitamin D [25(OH) D] has immune modulatory effects and plays a role in intestinal immunity. Low serum 25(OH) D < 15 ng/mL was associated with increased risk of CDI. This suggests vitamin D may have a role in determining susceptibility to CDI in outpatients.(102) Another study in adult patients to investigate whether pre-admission 25-hydroxyvitamin D (25(OH) D) levels are associated with the risk of hospital-acquired CDI, found vitamin D status before hospital admission was inversely associated with the developing CDI. These data support the need for randomized, controlled trials to test the role of vitamin D supplementation to prevent CDI.(103)

2.4.4 How does CD influence in the IBD outcome?

Although a considerable number of studies support a substantial increase in incidence, severity, and health care costs for Clostridium difficile infection (CDI) in inflammatory bowel disease (IBD), only a few have evaluated its impact on IBD outcome. Most studies showed that IBD patients with CDI present higher proportion of worse outcome than those without CDI. These patients had a longer length of hospital stay, higher rates of colectomies, and increased mortality. Patients with ulcerative colitis were more susceptible to CDI and had more severe outcome than those with Crohn's disease. (39, 40, 42, 43, 104-106)

Ananthakrishnan et al. (107) found that had an increase nationwide in CDI complicating IBD hospitalizations between 1998 and 2007. During 2004-2005, more than half of the infected IBD patients required hospitalization, and 20% required colectomy.(43) From 2005 to 2010, CD colitis had doubled in North America with an increase in the morbidity and mortality in IBD patients.(108)

CDI in patients with IBD associated with higher rate of recurrence (34%) and with higher morbidity than in the general population. Patients with IBD required more hospitalization (57%) and

escalation of therapy (67%) (immunomodulators/biologic treatments) following CDI, suggesting that CD increased severity of IBD.(31) Kaneko et al.(47) did not find association with any demographic factor or colectomy rate. However, CDI eradication therapy allowed some refractory patients to withdraw from steroids.

Several reported cases with infliximab therapy have provided favourable outcomes in UC patients with CDI, suggesting that infliximab treatment may be protective; however, the optimal infliximab treatment regimen for UC patients with CDI remains to be established.(29)

Most studies have focused on demonstrating that CDI is associated with adverse outcomes in IBD patients. However, few studies have attempted to identify predictors of severe outcomes associated with CDI in IBD patients: serum albumin <3 g/dL, haemoglobin < 9 g/dL and serum creatinine >1.5 mg/dL were independent predictors of severe outcomes in hospitalised IBD patients with CDI.(109) Often, there is no evidence of colonic changes with CD infection, including pseudomembranous exudate. However, a severe clinical course may occur, including toxic colitis and toxic megacolon. Hypervirulent CD strains have been reported raising concern for a more severe disease in IBD patients. Furthermore, small bowel involvement or CD enteritis has been increasingly described, usually in those patients with colectomy or total proctocolectomy for severe and extensive IBD. Furthermore, refractory or treatment-resistant pouchitis may occur with CDI.(18)

In a study from 2000 to 2008 most patients had a successful outcome and only one patient with UC needed a semi-urgent colectomy. The use of immunosuppressive drugs in IBD did not seem have a negative influence in the outcome but CDI appeared to be associated with escalation of medical therapy in the year following to the infection. (58) Moreover, CDI and severe disease on endoscopy seemed to be associated with an increased risk of subsequent colectomy on long-term follow-up.(110)

There is an apparently adverse outcome associated with the use of combined antibiotics and immunosuppressant treatment, particularly corticosteroids compared with antibiotics alone. Ben-Horin et al. (111) in a retrospective cohort study from European centres found 12% of patients with antibiotic treatment and immunomodulators presented: death or colectomy within 3 months of admission or in hospital, systemic complications, megacolon and bowel perforation compared with none of 51 given antibiotics alone. The treatment with one or more immunomodulators increased the risk of having an adverse outcome independent of disease severity at presentation (OR 17; 95% CI 3.2-91).

CDI has associated with worse outcome among hospitalized children, including increased risk of death. Vendetti et al. (112) identified risk factors for all-cause in-hospital mortality among children with CDI. Select chronic conditions and more severe diseases increased possibility of death.

In conclusion: CD can imitate an IBD relapse. Thus, it is essential that physicians are vigilant (58) Early suspicion is the best strategy to prevent complications related to CDI. In a recent study, Axelrad et al. (113) studied the use of nursing admission workflow to increase the rate of CDI diagnosis for inpatients with IBD with a relapse. There was a significant increase in the diagnosis and patients who received a test were more likely to have CDI, shorter hospital stays, and fewer readmissions.

2.4.5 May recurrent CDI be a severe problem as in no-IBD population?

Patients with IBD are at increased risk of developing C. difficile infection, have worse outcomes of CDI-including higher rates of colectomy and death, and experience higher rates of recurrence. In fact, clinical practice based on European, American, and Spanish guidelines recommend in all relapses, investigate in stools samples the presence of CD toxins in IBD out and inpatients but do not incorporate a section on this aspect. It would be important; we had a protocol with the best treatment strategies in this situation. We are going to review the novel and innovative treatment strategies to reduce treatment costs and prevent recurrence of CDI.

Despite effective antibiotic treatments, recurrent infections are common. Recurrence or relapse is not commonly due to development of antibiotic resistance of the CD, but it is considered to be due to a defective host immune response, persistence of spores in the gastrointestinal lumen after discontinuation of antibiotics, failure to re-establish intestinal flora or to re-infection by a new strain of CD. (114)

Humoral immune response as a predictor of recurrence in Clostridium difficile infection. Low serum concentrations of antibodies directed against the toxins TcdA and TcdB have been associated with a higher risk of recurrence of CD after successful antibiotic treatment. However, there are conflicting reports. Bauer et al.(115) compared serum levels of antibodies of patients with a single episode with those of patients who suffered a recurrence. They found that: older age, comorbidity, immunocompromised state and low serum levels of anti-TcdA and anti-TcdB antibodies were associated with recurrence. However, serum levels of antibodies directed against cell surface antigens were not. Serum TcdB-neutralizing capacity, which correlated only weakly with serum IgG anti-TcdB, was not significantly associated with recurrence. In another study, found that CD recurrence was characterized by pro-inflammatory peripheral blood mononuclear cell (PBMC) phenotype. Used flow cytometry to define inflammatory (Th1 and Th17) and regulatory [Foxp3(+) T-regulatory (Treg)] cells present in circulating peripheral blood mononuclear cells (PBMC) from CDI patients.(116) A better understanding of intestinal microbiota and its role in CDI has opened the door to this promising therapeutic approach such as FMT that thought to resolve dysbiosis by restoring gut microbiota diversity thereby breaking the cycle of recurrent CDI.(117)

The recurrence of CDI, in general, the population can be a severe problem because about 25% of those suffering from CDI have a recurrence after the first course of treatment. For those patients with a recurrent episode of infection, there is a 40% chance of experiencing another recurrence and for those who have had more than 2 episodes there is a 60% chance of a further episode.(118) Global spread of the 027 (BI/NAP1/027) ribotype. C. difficile cases attributed to ribotype 027 strains had high recurrence rates (up to 36 %) and increased disease severity. Moreover, CDI recurrence was associated with excessive costs, which were mostly attributable to a significantly longer overall length of stay.(119) Prognostically unfavourable signs of complicated CDI with ileus, toxic megacolon, perforation, or sepsis (less than 5% of cases) include the absence of colonic peristalsis, sudden-onset constipation, extreme leucocytosis, and high fever. Mortality resulting from CDI depends on the severity of symptoms, underlying diseases, and age. It ranges from 3% to 14%. Relapses occur in approximately 20% of cases following completion of initial treatment, typically within the first 2 to 6 weeks in patients with risk factors. The 30-day attributable mortality rate was 6.9 percent.(24)

What about IBD patients? CDI in IBD patients resulted in 34% recurrence, required more hospitalization and escalation of therapy, suggesting that CD resulted in increased severity of IBD disease.(31) Moreover, as in the general population, the recurrence of CDI after successful initial treatment can be a significant problem. On average, an initial recurrence can be expected in 20-30% of cases and after another relapse may occur in up to 65% of patients. Risk factors associated with recurrence include older age (greater than 65), longer hospital stays (greater than 16 d), the presence of comorbidities and another course of antibiotics. Therefore, the severity of recurrent episodes of CDI cannot be underestimated.

The new epidemic strain (027) has been associated with a higher rate of recurrence about 47% due to a low Ig G against toxin A due to having an inadequate antibody response. Pepin et al.(120) reviewed the outcomes of a first recurrence of CDI with the epidemic strain during the Quebec outbreak in 2005 and found 11% of these patients had at least one severe complication such as shock, colectomy, megacolon, perforation or death within 30 days. Complicated recurrence CDI was strongly associated with three factors: older age (>65), elevated white blood cell count (> 20.000) and renal failure. The long-term negative impact of CDI was also investigated by Musheret al.(121) finding 22% of patients developed recurrent diarrhoeal disease more than 90 days after the initial episode and completed the treatment, 83% of whom were toxin positive. Recently, Deshpande et al.(122) have published a systematic review and meta-analysis to evaluate current evidence on the risk factors for recurrence CDI (rCDI). Approximately, 20-30% of patients with primary Clostridium difficile infection (CDI) develop recurrent CDI within 2 weeks of completion of therapy. The mechanism of recurrence remains unknown, but a variety of risk factors has been studied. Abou et al.(123) studied risk factors for recurrence, complications and mortality in Clostridium difficile infection: a systematic review. Laboratory

parameters currently used in European and American guidelines to define patients at risk of a complicated CDI are adequate. Strategies for the management of CDI should be tailored according to the age of the patient, biological markers of severity, and underlying co-morbidities. 68 studies were included: 24 assessed risk factors for recurrence, 18 for complicated CDI, 8 for treatment failure, and 30 for mortality. Older age, use of antibiotics after diagnosis, use of proton pump inhibitors, and strain type were the most frequent risk factors for recurrence. Older age, leucocytosis, renal failure and co-morbidities were frequent risk factors for complicated CDI. When considered alone, mortality was associated with age, co-morbidities, hypo-albumin, leucocytosis, acute renal failure, and infection with ribotype 027.

About 12% of hospitalized children with CDI had recurrent disease. The independent risk factors associated with recurrent disease were: concomitant antibiotics and community-associated. The authors recommended that antibiotics should be discontinued whenever possible in case of CDI in this patients (124). Recurrent CDI in children was 22%, and it was significantly associated with recent surgery, malignancy, and the number of antibiotic exposures. (56) In another study in children in and out-patient, risk factors for recurrent Clostridium difficile infection were malignancy and tracheostomy tube dependence (125).

Hikone et al. (82) found that intensive care unit hospitalization and malignancy were risk factors for recurrent CDI. They recommended that these patients should be carefully monitored for recurrence and provided with appropriate antimicrobial stewardship.

A Spanish study in an ICU found that less than 1% of the patients admitted to a clinical-surgical ICU developed CDI, but a high risk of recurrence/complications was associated with prolonged ICU stay. There is a lack of studies on the incidence of CDI in European ICU outside the context of outbreaks. (126)

Recurrence of CDI among veterans with spinal cord injury and disorder. Concomitant fluoroquinolone use was a risk factor. In contrast, tetracycline and cerebrovascular accident were protective. The length of stay greater than 90 days from the initial CDI episode was also a risk factor for recurrence. CDI.(127)

Hu et al.(128) aimed to develop a prediction rule for recurrent CDI to identify high-risk patients. The clinical prediction rule included: age>65 years, severe or fulminant illness (by the Horn index), and antibiotics use. A second rule combined data on serum concentrations of immunoglobulin G (IgG) against toxin A with the clinical predictors. The study validated a clinical prediction rule for recurrent CDI that is simple, reliable, and accurate and can be used..

Prevention of recurrent CDI is a therapeutic challenge, but the treatment of CDI can be hard. Vancomycin and metronidazole have been used in the treatment of CDI. However, it remains unclear why patients are at risk of treatment failure and recurrence. Antibiotics and PPIs should be discontinued during CDI treatment to increase therapeutic efficacy, and the use of anticancer treatment and corticosteroids should be delayed as long as possible after patients are cured to prevent recurrence (129). The hypervirulent strain of CD has higher rates of recurrence, increasing severity and mortality. Faecal microbiota transplantation (FMT) could be an alternative treatment for recurrent CDI in IBD patients too.

2.5 How can we diagnose CDI in IBD patients?

2.5.1 Laboratory test

The best laboratory diagnostic approach to detect CDI is not clear. The diagnosis of CDI requires the detection of toxigenic CD or its toxins and a clinical assessment (130). Detection of toxin in the stool may be a better predictor of CDI disease and severity.(131) Shimizu et al. (76) found that in the cases of CDI were detected by the initial screening test were more severe than those where the toxins were not detected at the initial screening but were identified by the culture. Until now, the diagnosis has been typically established by *testing toxins A/B in stool by enzyme immunoassay (EIA)* in a patient with diarrhoea. The EIA for toxin A/B is used by most clinical laboratories because it is fast, convenient and inexpensive. However, this strategy is unsatisfactory because of its low sensitivity resulting in significant false negatives. Due to concerns about the poor sensitivity of toxin ELISAs, which can range from 50–95%, physicians commonly repeat testing in patients with persistent diarrhoea if prior stool specimens tested negative. However, toxin ELISAs are not 100% specific, and false positive results can occur. The false positive rate can increase, with a resulting drop in the positive predictive value, when testing is in populations with a low prevalence of CDI or if the specificity of the assay decreases. (132, 133) Recently, Song et al.(134) have developed ultrasensitive digital enzyme-linked immunosorbent assays (ELISAs) for toxins A and B using single-molecule array technology. This method can provide a rapid and simple tool for the diagnosis of CDI with both high analytical sensitivity and high clinical specificity.

Deshpande et al.(135) studied whether repeat stool testing improved the diagnosis of CDI in hospitalized IBD patients compared with hospitalized patients without IBD The first stool sample tested was positive in 81% of patients. Successive second and third stool samples were positive 14%, and 5%. Thus, there were minimal diagnostic gains of repeat testing by EIA or CRP in patients without IBD.

Nowadays EIA for toxin A and B is not recommended due to its low sensitivity, and it has led to a search for more accurate test methods (136). Goldenberg et al. (137) investigated the performance of a two-step algorithm for diagnosis of CDI using detection of glutamate dehydrogenase (GDH). GDH-positive samples were tested for C. difficile toxin B gene (tcdB) by polymerase chain reaction (CRP). Screening for GDH before confirmation of positives by CRP is cheaper than screening all specimens by CRP and is an effective method for routine use. The detection of GDH in the stool is fast (15-45 min), convenient, inexpensive, and sensitive. GDH is a common antigen expressed at high levels by all CD strains. However, the test only documents the presence of CD, but not the presence of a toxigenic strain (20% of CD strains do not produce toxin) or the presence of toxin in stool. Moreover, GDH (+) stool requires confirmation of toxin production with a second test. (137) Therefore, a two-step method is recommended as the most appropriate approach. (76) Moreover, it permitted the resolution of most cases on the day of arrival, reducing the number of unnecessary or missing isolations. (138) Shimizu et al. (76) found the cases of CDI in which the toxins were detected by the initial screening test were more severe than those where the toxins were not but were identified by the toxigenic culture. Also, the most significant factors affecting the severity score were an older age and a lower serum albumin level.

Stahlmann et al. (22) found that the multiplex CRP was faster and more sensitive compared with culture and allowed identification of hypervirulent strains on the same day. Krutova et al. (139) recommended when GDH positivity and A/B toxin negativity that if no confirmation test were available, the result should be considered as epidemiologically and clinically significant, if other causes of diarrhoea are ruled out

In last years, a high sensitive polymerase chain reaction (CRP) test for the toxin B gene of CD (detects low copy numbers of a toxin gene in CD) is increasingly used to diagnose. Nevertheless, positive CD CRP results occur with similar frequency in IBD patients with and without active disease. Therefore, a positive result may reflect colonization in a subset of patients with IBD, confounding clinical decision making in the managing of disease exacerbations. (140) CRP based assays and combination Elisa algorithms have improved the sensitivity and specificity of testing, to detect CD colonization. At present, polymerase chain reaction (CRP) has increasingly replaced toxin A & B enzyme immunoassay (EIA) for testing of CDI. CRP may increase CDI incidence rates by greater than 50%. Some authors had noted an increase from 6.5% positive samples before the use of CRP to 15%. Also, the CRP cannot be used for suspected relapse as up to 56% of patients will be positive by CRP at 1-4 week after completion of therapy. However, despite its high sensitivity and specificity, the positive predictive value may be only 63%. Leibowits et al. (141) found C. difficile CRP assays are frequently positive in hospitalized children both with and without diarrhoea. They observed a high level of toxigenic C. difficile colonization in children, suggesting that a positive C. difficile CRP result in a child with diarrhoea should be interpreted with caution. Akbari et al. (142) in a recent study, found the number

of tests performed decreased and proportion of positive increased since CRP introduction. CDI incidence remained constant. Only found that albumin and inflammatory bowel disease status differed between the EIA and CRP. The length of stay was shorter in the CRP group. Thus, an earlier detection and quicker onset of therapy determined a less severe disease. Mortality did not change since CRP introduction.

The *underdiagnosis* of CDI is a severe problem in Europe. It was performed a study to measure the underdiagnosis of CDI across Europe (EUCLID). (143) It was the largest scale study in Europe. This European, multi-centre, prospective bi-annual point prevalence study of Clostridium difficile Infection in Hospitalized patients with diarrhoea (EUCLID) will allow professionals to measure the true rate of CDI accurately. Aims were to investigate how common CDI was among hospitalized patients with diarrhoea in 20 countries and 500 hospitals all over Europe. It should give an accurate vision of the under-diagnosis and under-testing in Europe. Only two-fifths of hospitals used an optimum methods for testing of CDI defined by European guidelines. The absence of clinical suspicion and suboptimum laboratory diagnostic methods means that an estimated 40.000 inpatients with CDI are potentially undiagnosed every year in 482 European hospitals. A previous study carried out in Spain in 2008 revealed that almost two-thirds of cases of CDI were misdiagnosed or not picked up at all, indicating very low clinical awareness of CDI within the hospitals.

For performance a diagnostic test, so it is important a high level of suspicion. Krishnarao et al. (144) found that a low testing rate made difficult a diagnosis although CDI prevalence was high, producing a delay in the CDI diagnosis. They found in their study that the testing for CDI was significantly lower than expected at diagnosis of IBD in spite of the prevalence of CDI among tested patients was 5%.

Recently, González-Abad et al. (145) studied the simultaneous detection of GDH and toxin A/B combined with CRP recovered undiagnosed cases of CDI. At the end of their study, they proposed an algorithm that could have a better cost-benefit ratio: a two-step algorithm: detection of GDH and CRP (in samples GDH positive).

What about repeat testing for CDI?

The 2013 ACG guidelines make a recommendation that repeat testing not is performed. However, recently, Aldrete et al.(72) recommended that repeat testing for CD CRP should take into consideration patients who may be at high risk for short-term acquisition. Repeat testing resulted in 4.5 % to 9.3 % extra positives without significant difference. Repeat sampling and multimodality testing may be chosen in an outbreak situation to detect all cases, effectively controlling nosocomial spread.(146)

Living et al.(132) studied the utility of repeat testing if the first one was negative. They concluded that specificity was as important as sensitivity when testing for CDI. Repeat testing for C. difficile should be performed with caution. They concluded that repeat testing should not be routinely performed because the decrease in positive predictive value that occurs decreases the usefulness of a positive result, and may result in harm to the patient. Thus, it is not recommended the practice of repeat testing unless there is a high index of suspicion and the results could alter patient management. They suggested that positive tests on repeat testing are false ones. They do not recommend repeat testing for C. difficile because of false positive tests may result in a negative impact on the patient.

General recommendations from American clinical update in 2014 (147)

The concern with EIA for toxin A/B, CRP and GDH as diagnostic tests have done an diagnostic approach similar to HIV and syphilis testing. The two-step method is recommended as the most appropriate approach.(76)

An American clinical updates in 2014 (147) recommended "multistep diagnostic procedures, combining a sensitive screening test with a confirmation test for the toxigenic infection." Only symptomatic patients should be tested. Repeat stool samples are not usually required. Cultures are not adequate to acute diagnosis.(147)

They proposed the following diagnosis approach: "(1) if CDI is suspected on clinical grounds, perform C. difficile testing according to your hospital laboratory protocol. (2) if the test is positive, continue or initiate treatment, if not started empirically; and (3) if the test is negative, make a clinical decision on whether to treat based on the likelihood of CDI (recent exposure to antibiotics or prior CDI, elevated white blood count or elevated creatinine or decreased albumin, age or other risk factors). If CDI is still suspected after a negative test, empiric treatment is reasonable. Repeat testing yields minimal additional true positives and increases cost". The ACG Guidelines make a recommendation that "Repeat testing should be discouraged."

It has investigated new diagnostics approach

Bomers et al. (148) in 2014 performed a very interesting study where they assessed the diagnostic accuracy of *a trained detection dog* for detecting CDI cases on Dutch university hospital wards in an outbreak setting. The dog's response was compared to the clinical diagnosis, supported by laboratory results. A trained detection dog can accurately detect CDI in hospitalized patients during an outbreak. Moreover, another study was performed again by Bomers et al. (149) who studied *an accurate,*

fast, and on-site detection of C. difficile (FAIMS). It is a portable mass spectrometry instrument that quickly analyzes the chemical composition of gaseous mixtures. It can differentiate between C. difficile-positive and -negative samples with high diagnostic accuracy.

2.5.2 Biological parameters

Laboratory parameters used in European and American guidelines are useful to define patients at risk of a complicated CDI. Strategies for the management of CDI should be done according to the age of the patients, biological markers of severity, and underlying co-morbidities. Leucocytosis, hypoalbuminemia, and elevation of baseline serum creatinine are highly suggestive of CDI. The elevated white blood cell (WBC) count is common (50%-60%), as well as increased band forms (47%) and may be marked elevated. The elevation of WBC may precede the onset of diarrhoea or abdominal discomfort and may be responsible for up to 58% of cases of unexplained leucocytosis in hospitalized patients. In a series of a patients with leucocytosis who were C. difficile toxin negative, empiric treatment for CDI led to resolution of leucocytosis. Furthermore, CDI can result in a protein-losing enteropathy with resultant hypoalbuminemia. Serum albumin of < 2.5 or a fall in albumin of > 1.1 have been associated with a poor prognosis (123)

2.5.3 Faecal markers

Toxins A and B produce a damage to intestinal mucosa and an inflammatory response secondary. This inflammatory response plays a role in how quick the disease progresses to colitis. Moreover, this inflammatory response influences to develop pseudomembranous colitis, which is life threatening if left untreated. This results justify the study of faecal markers.

One of them is faecal lactoferrin (typically used as an indicator of inflammatory bowel disease activity tests). Levels of faecal lactoferrin, which is released from the secondary granules of faecal leucocytes, and other inflammatory markers rise significantly in patients with severe CD disease compared with levels in patients with a milder case of the disease. However, this inflammation marker lack sensitivity and specificity and add little to the diagnostic evaluation. (150) These tests express the degree of intestinal inflammation and provide valuable information about the necessity of starting early with the antibiotic treatment. The presence of faecal leucocytes in patients with hospital-acquired diarrhoea is associated with CD. (74)

Nowadays, there are not studies with faecal calprotectin. The measure of faecal calprotectin can add information related to the severity of the CDI, in the follow-up to evaluate the response to the

treatment with the monitorization of its levels and recurrence, but it is not useful for doing the diagnosis due to nonspecific as faecal lactoferrin.

2.6 Is the treatment the same as general population?

2.6.1 Overview

Management of CDI in IBD patients with a relapse has not been optimised. We do not have specific guidelines for management of this infection in IBD patients until now. It is contradictory that, all IBD workgroups recommend the investigation of CD in stools sample in all resistant relapses to conventional treatment in outpatient and all inpatients, but there are not specific recommendations for the treatment CDI in these patients specifically. The elaboration of a specific therapeutic guideline in IBD would be important to improve the treatment of this infection in Spain and Europe.

For that reason, we have to follow the indications of Microbiology General Guidelines for CDI treatment, in general, population. In 2009, the first European Society of Clinical Microbiology and Infection (ESCMID) treatment guidance document for CDI was published and has been applied widely in clinical practice. (151) Latest ESCMID Guidelines have been published in 2014. (152) In particular, after the recent development of fidaxomicin, new alternative drugs for the treatment of CDI in the USA and Europe, there has had an increasing need for an update on the comparative effectiveness of the currently available antibiotic agents in the treatment of CDI, thereby providing evidence-based recommendations on this issue. The recommendations to improve clinical guidance in the treatment of CDI, are specified for various patient groups such as non-severe disease, severe CDI, first recurrence or risk for recurrent disease, multiple recurrences and treatment of CDI when oral administration is not possible. The options include: antibiotics, probiotics, and faecal or bacterial intestinal transplantation. The antibiotics recommended: metronidazole, vancomycin, and fidaxomicin. Faecal transplantation is recommended for multiple recurrences. We are going to revise the most important therapeutic recommendations. (*See Appendices: Tables 1-10 with the most important recommendations related to treatment CDI*)

There is not agreement among gastroenterologists on whether combination antibiotics and corticosteroids or antibiotics alone should be given to IBD patients with CDI-associated relapses. Treatment modalities for CDI have not been examined in randomised clinical trials in the IBD population. Newer antibiotics, immunotherapy, and faecal microbiota transplantation may alter current treatment strategies. (40) It would be important we had specific protocols for the best treatment strategies in IBD patients specifically. Thus, controlled trials are needed to investigate the optimal management approach to this clinical dilemma. (153)

Prophylactics and hygienic measures are the same as in the general population: hand washing and cleaner hospital environments, reduce the risk of acquiring and spreading this preventable infection.

In the review of this issue, we are going to start showing the limitation of the evidence about the specific treatment for CDI in IBD patients, trying to show a perspective on what could be future researches. We are going to show *several gaps*:

- There are no controlled therapeutic trials of CDI in IBD and we need prospective multicentre ones to improve our understanding of the impact of CDI on IBD patients and define appropriate therapeutic regimens to improve patient outcome.

- In IBD patients, vancomycin appears to be more efficacious than metronidazole. Randomised controlled trials are required to define clearly the appropriate management for CDI in this patients.

- IBD with CDI is frequently treated with a combination of antibiotics and immunomodulators. However, this combination tends to associate with a worse outcome than antibiotic therapy alone. Prospective controlled trials are needed to optimise the management of these patients. Could we control relapses only with antibiotic treatment? One of the main points of treatments in IBD is to save corticosteroids.

- Clinical studies are required to evaluate efficacy of vancomycin vs fidaxomicin.

- Does CDI get worse with immunomodulators and biological treatments? Is CDI a cause or an effect of immunosuppressant and biological treatments?

Metronidazole/Vancomycin

Despite numerous treatment trials for CDI, dating back to 1978, the drug of choice for CDI remains controversial. In fact, Pepin et al. (154ic) said that “there are few common infectious diseases in developed countries for which the treatments used in 2006 are essentially the same as those recommended one-quarter of a century ago”. However, in my opinion, the things are changing now. A clear example is the recent creation of the first faecal bank in the USA to treat recurrent and refractories CDI.

In general, metronidazole and vancomycin are most used drugs used to treat CDI. A few changes have occurred in the treatment of CDI over the last 30 years. Fortunately, some recent studies have not revealed resistance to the main antibiotics for its treatment. Patients with mild or moderate diarrhoea are

treated with metronidazole 500 mg TID for 10 to 14 days. Patients with complicated CDI are treated with vancomycin 125 to 250 mg QID for 10-14 days. Moreover, switching to vancomycin in patients who do not improve within 72 hours of initiation of treatment with metronidazole could be appropriate. Adjunctive therapy can be done with vancomycin enema 500 mg in 100 ml saline and intravenous metronidazole. Surgery with total colectomy is indicated in severe colitis with systemic symptoms.

An additional challenge facing CDI treatment in IBD patients: the decreased efficacy of metronidazole and the need for oral vancomycin in hospitalized patients. As a general population, vancomycin and metronidazole appear to have similar efficacy in mild or moderate episodes but vancomycin is preferred for severe disease. Furthermore, early surgical consultation is recommended in patients with severe disease for improving outcome. (109)

Metronidazole does not seem to have much resistances. In a recent study, performed from nosocomial and community-acquired CDI between 2008 and 2010 all strains studied were susceptible to metronidazole. When they comparing their results with others earlier findings from 2006 to 2007, metronidazole susceptibility did not show changes (155). Again, in 2008 it was spoken about metronidazole resistant o failure, Hu et al. (156) attempt to identify risk factors for metronidazole failure. They did not find differences in metronidazole failure rates in 1998 and 2004 to 2006 but the patients with recent cephalosporin use, CDI on admission, and transfer from another hospital were more likely to metronidazole failure.

Vancomycin

Therapeutically, oral vancomycin has emerged as superior treatment for IBD patients with severe disease, including those who require hospitalisation and metronidazole for mild or moderate infection. Patients with UC and non-severe CDI have fewer readmissions and shorter lengths of stay when treated with a vancomycin compared with those treated with metronidazole alone. Patients with ulcerative colitis and CDI should be treated with vancomycin (157). In general, for CDI treatment in IBD patients, vancomycin appears to be more efficacious than metronidazole. Randomised controlled trials are required to define clearly the appropriate management for CDI in patients with IBD.

Unfortunately, vancomycin capsules are very expensive. Most hospitals using the generic intravenous formulation and compounding it in water as a liquid vancomycin solution. Thus, 500 mg of vancomycin powder are reconstituted in 20 cc of water (often with flavouring to hide the bitter taste of vancomycin). Stability of the vancomycin solution in the refrigerator (4 degrees C) is at least 75 days and at least 26 days at room temperature (25 degrees C). (158)

Immunosuppressant treatment

In IBD patients with CDI is controversial whether immunomodulators or corticosteroid therapy for IBD should be continued in patients with CDI is controversial. Normally, if the patient was treated with immunosuppressors or biologics we would not stop it. When CDI is treated in the patient was treating with immunomodulators or biological treatments these do not remove. However, limited data suggest that co-administration of immunomodulators (IM) with antibiotics (AB) result in a worse outcome than antibiotic therapy alone but we do not have studies prospective that had studied this point. Ben-Horin et al.(111) investigated the effects of combined therapy with antibiotics and immunomodulators in patients with IBD and CDI and found that this combination produced a worse outcome compared with antibiotic therapy alone.

Yanai et al.(153) investigated the prevalent practice among North American gastroenterologists in treating relapses in IBD patients with CDI: antibiotics alone or combined antibiotics/immunomodulators. The rate of administering combined antibiotics and immunosuppressant treatments was similar for the IBD experts and the non-IBD experts. 11% of gastroenterologist withdrew maintenance azathioprine upon the diagnosis of CDI. More IBD experts stopped azathioprine treatment compared with the non-IBD experts. Overall, 65% of gastroenterologists said that they believe these patients were affected by two simultaneous processes. There is not agreement among gastroenterologists on whether the combination antibiotics and immunosuppressant treatment or antibiotics alone should be given to IBD patients with CDI-associated relapses. Controlled trials are needed to investigate the optimal management approach to this clinical situation. We need controlled trials to optimize the management of these patients.

Biologic agents

The use of biologic agents does not seem to increase the risk of acquisition of CDI in IBD patients, on the contrary, biologic treatment could be useful to treat these patients. Seicean et al. (29) presented a case report of worsening UC in the presence of recurrent CDI, and infliximab therapy provided favourable outcome, suggesting that infliximab treatment may be protective; however, the infliximab treatment regimen for UC patients with CDI remains to be established.

2.6.2 Treatment decision based on stratification by disease severity

In the comparative studies with vancomycin and metronidazole did not stratify the patients by severity of the disease. Recently, a scoring system has been developed in the treatment of CDI. This score allows the physician to determine which patients are at highest risk for severe CDI. It was started

by Pepin et al. (154) who developed local recommendations, because of the devastating epidemic in Quebec caused by the new epidemic B1 strain. In January 2004, they developed local recommendations for the use of oral vancomycin: a WBC greater than 20000 cells/mm3 and a serum creatinine greater than or equal to 200 μmol/L. This recommendation was based upon a reduction of complicated CDI by 79% if vancomycin was the initial treatment compared to metronidazole (159). Zar et al. (160) conducted the first randomized, double-blind, placebo-controlled trial comparing metronidazole and vancomycin in the treatment of CDI that stratified patients based on the severity of the disease. The authors concluded that metronidazole and vancomycin was equally effective for the treatment of mild CDI; however, vancomycin was superior for treating patients with severe CDI.

Strategies for CDI management should be done according to the age of the patient, biological markers of severity and underlying co-morbidities. (123). S*evere CDI* (152) was defined as an episode with (one or more specific signs and symptoms of) severe colitis or a complicated course of disease, with significant systemic effects and shock, resulting in ICU admission, colectomy or death. One or more of the following unfavourable prognostic factors can be present without evidence of another cause: marked leucocytosis (> 15 10^9/ L), decreased blood albumin (< 3 gr/L) and rise in serum creatinine level (>1.5 times the premorbid level)

Miller et al. (161) created the ATLAS score for CDI, which predicts the response to therapy. They found that a combination of five clinical and laboratory variables (age, treatment with systemic antibiotics, leucocytes count, albumin and serum creatinine as a measure of renal function) measured at the time of CDI diagnosis were useful to predict treatment response. This scoring were able to predict treatment response.

An early surgical consultation is key for improving outcome of patients with severe disease. (109) Surgical intervention is rarely required but in patients with severe disease or clinical deterioration, early total colectomy or loop ileostomy may be life-saving. The outlook for patients requiring surgery remains poor. (162)

When oral treatment is no possible, parenteral metronidazole is recommended, preferably combined with intracolonic or nasogastric administration of vancomycin. (152)

2.6.3 What is the best approach in the recurrences?

The recurrence of disease after therapy remains a problem. The treatment includes general measures such as supportive care and infection control measures.(163) Another main goal of the therapeutic approach is treatment and the prevention onset new infections and recurrences. It should use

vancomycin earlier and start with new treatments such as faecal transplant in severe/recurrent episodes (152) (*see Appendices: Tables 6, 7 and 8*)

The initial response to metronidazole and vancomycin is approximately 60%, with subsequently decreased response with subsequent relapses. Persistent alterations in the intestinal microbiota decrease natural colonization barriers and increase the risk of relapse of CDI with the same, or different strain. Several reports since 1958, amounting to over 325 cases, including one systematic review, have described high cure rates of recurrent CDI with faecal transplantation performed via retention enema, colonoscopy, or nasogastric tube. (164) Brand et al.(165) in a study in 2012 demonstrated that cure rates were sustained over long-term follow-up of a mean of 17 months.

Patients must be treated with metronidazole or vancomycin. They usually respond to antibiotic treatment, but the therapy affects to normal bacterial flora. Thus this treatment could predispose to recurrent CDI. The colon damaged seems to be more susceptible to reinfection. Relapses occur approximately in 20% of the patients. (136)

Most authors recommended, repeating the treatment with the antibiotic used in the first time, usually metronidazole, but this is not recommended in the latest guidelines. (152, 166). For more than one recurrence, it is recommended a combination of a prolonged taper of antibiotic with oral vancomycin, followed by pulsed dosing. (152) The pulsed dosing of vancomycin allows time for germination of residual spores during the days of antibiotics, vegetative form disappear when the antibiotic is given again. It is recommended: vancomycin 250 mg every 2 or 3 days for 3 week. (167, 168)

2.6.4 Role of FMT in the treatment of recurrent and refractory CDI

Altered microbiota as a potential target for therapy is a growing focus on investigation because of its potential to enhance the resistance to infection and to reduce inflammatory diseases. Most extreme manipulation of the intestinal microbiota is related to faecal microbiota transplantation (FMT) from healthy donors to individuals with specific diseases. Although the concept of faecal transplantation has become more widely practiced in recent years, it has a long history. More than 2000 years ago Ge Hong used FMT to treat food poisoning and severe diarrhoea. Fabricius of Acquapendente in the 16th century described the transplantation of enteric bacteria. In recent time, the first reports about the use of FMT in our traditional medicine was done by Eiseman who treated 4 cases of pseudomembranous colitis with faecal enema obtained from a healthy donor. (2) Since the first reported use of FMT for recurrent CDI in 1958, systematic reviews of case series and case report have

shown its effectiveness with high-resolution rates compared to standard antibiotic treatment. FMT is administrated by naso-jejunal or colonoscopy for the treatment of recurrent CDI. Restoration of intestinal flora and restoration of colonization resistance are the mechanism responsible for treating in these recurrent episodes of CDI

Faecal microbiota transplantation (FMT) is considered a successful therapy for recurrent and refractory Clostridium difficile infection (CDI) based on recent clinical trials. FMT restores essential components of the microflora that could reverse the inflammatory processes observed in IBD so it may be beneficial for the treatment of ulcerative colitis and Crohn's disease, particularly those with concurrent CDI or with pouchitis (2, 26, 164). However, there are to perform more studies to evaluate its useful in IBD patients. (169)

There are studies in children to treat recurrent Clostridium difficile infection via nasogastric tube or colonoscopy without adverse events during short- or long-term follow-up, and all of the patients had clinical improvement of gastrointestinal symptoms.(170) Hourigan et al. (38) studied CD eradication and microbiome changes in children with and without IBD after treatment with TMT. The authors concluded that FMT was effective for CDI in children with and without IBD. In those with IBD, bacterial diversity returns to pre-FMT baseline 6 months after treatment, suggesting IBD host-related mechanisms modify faecal microbiome diversity. The long-term consequences of FMT with regards to infection, cancer, autoimmune and metabolic diseases, are not known.(26)

Recent research about commensal microbes and their impact on the host will lead to the development of new probiotic agents.(171) The ultimate goal is the development of powerful probiotic regimens that can replace FMT. Currently, FMT should only be given in a strict experimental setting for other conditions than CDI.(28)

Some questions remain unanswered: the optimal protocol for donor faeces administration (naso-duodenal tube, enema or colonoscopy) is unknown. Furthermore, the efficacy of this modality in severe CDI, as well as in special populations such as patients with inflammatory bowel disease, cirrhosis, and immune compromised states. Further studies are required, however, to determine the optimal protocol for donor faeces administration. (UEG: Vienna, October 2014) It is a safe and effective treatment for recurrent CDI and is now recommended in European treatment guidelines. Healthy microbiota harvested from a donated stool sample is transplanted into the intestine by colonoscopy or enema-where it helps to restore the normal composition of the gut flora and overcome the harmful consequences of CDI. It eradicates recurrent infection in around 90% of patients.

2.6.5 What is coming up: future treatments

In a briefly way, we are going to revise some of most new treatments for CDI although is not our main goal. In the treatment of this infection as important as treat the first episode is to avoid the recurrences. Several novel therapies are currently under study: new antimicrobial agents have a good activity against CD without altering normal gut flora, CD toxin-absorbing compounds, and antibodies and vaccines against CD toxin (immunotherapy) and faecal microbiota transplantation may alter current treatment strategies

Fidaxomicin

Patients responded to oral vancomycin or metronidazole; however, the rate of recurrences is high. For that reason, it has investigated new therapies to treat this infection. Fidaxomicin, first-in-class macrocyclic antibiotic has minimal intestinal absorption, high faecal concentration, and cause less disruption of anaerobic microbiota during treatment of CDI than vancomycin and moreover, it has activity against many vancomycin-resistant enterococci. (172) Recommended dose is 200 mg every 12 hours for 10 days. In Spain, we already have it but the main problem is its high cost.

In two double-blind, non-inferiority, randomised controlled trial, comparing the efficacy and safety of fidaxomicin with vancomycin in the treatment of CDI. The authors demonstrated fewer recurrences and higher rates of sustained clinical cures compared with the current gold standard, vancomycin. Thus, fidaxomicin can be an alternative treatment for CDI, with similar efficacy and safety to vancomycin. (173) This drug is an antibiotic therapy for CDI and must be considered as a first-line agent for patients with risk factors known to have a relapse and severe infection. (174) A study in 2011 assessed the efficacy of fidaxomicin versus vancomycin as therapy for CDI in individuals was taking concomitant antibiotics. (175) Due to low recurrence ratio and safety, it could be one of the first lines of CDI treatment in patients with cancer. (176)

Recently, Chilton et al. (177) found that pulsed or tapered regimens of fidaxomicin had the same efficacy of vancomycin but reduced the risk of recurrent CDI compared with vancomycin. Moreover, pulsed or tapered regimens of fidaxomicin allowed greater bifidobacteria recovery than the extended (20 days) regimen. Consequently, this may enhance suppression of C. difficile while allowing microbiota recovery; clinical studies are required to ascertain the potential of this approach in reducing recurrent CDI.(178)

Probiotics

Probiotics are used by everybody, but we do not have solid scientific evidence. Could probiotics have a protector role? Recently, Ramakrishnan et al.(179) performed a study in India where there is an overuse of antibiotics to demonstrate an increase of CDI. However, they found that the incidence of CDI in India (1.67%) was no different from reported in the USA (1.6%) using similar techniques of detection (polymerase chain reaction test). They offer a possible explanation for this paradox. It is likely that a diet rich in fibre, yogurt, and possibly turmeric may have a protective role in decreasing the incidence of CDIs in India

Most gastrointestinal disease specialists recognize a role for probiotics and have used it as part of their therapeutic options. Williams et al.(180) in 2010, demonstrated that all physicians believed probiotics were safe for most patients, and 98% responded that probiotics had a role in treating gastrointestinal illnesses or symptoms, and 93% of physicians had patients taking probiotics most often for irritable bowel syndrome.

Differences of pathogen clearance and microbiome alteration during treatment of CDI appear to explain treatment outcome. The hypothesis that probiotic microbes (Lactobacilli) could help to prevent the CDI is supported by the observation of persistence of Lactobacilli during and after treatment of CDI. (181) In conclusion: the use of conventional probiotics remains controversial, and most studies are of low quality. No overall recommendations can be provided by the moment.

Active and passive vaccination

There are good data from animal experiments. Current research on vaccination is at the stage of phase III clinical trials. It has to investigate which population sample would be more effective to give the vaccine. There are studies on going.

SMT19969

It is a new antimicrobial agent against 162 strains from 35 less frequently recovered intestinal Clostridium species. Currently under development for the treatment of CD, it has been reported the results from a phase I study (182) comparing in vitro activities of SMT19969 and its implications for treating Clostridium difficile recurrence. SMT might have less impact on normal gut microbiota than other CDI treatments. (183)

LFF571

Novel thiopeptide antibacterial that shows in vitro potency against CD comparable or greater than other clinically used antibiotics. It was compared the pharmacokinetics of LFF571 and vancomycin in patients with moderate CDI as part of an early efficacy study, and the results seem consistent demonstrating the retention of LFF571 in the lumen of the gastrointestinal tract. (184)

Amixicile

Another novel treatment that shows efficacy in the treatment of CDI and recurrences, but still in a mouse models. It is a water-soluble derivative of nitazoxanide (NTZ), an antiparasitic therapeutic that also shows efficacy against CDI in humans.

2.7 General recommendations for the prevention and control of CDI

In developed countries, Clostridium difficile is the most common cause of healthcare-acquired infection. CDI is a frequent cause of morbidity and even death. It also produces huge economic costs, because of infected patients with CDI stay in the hospital for some additional 1-3 weeks more than normal. Regarding cost and productivity, C. difficile is a major burden on our health care system.

The key points for the prevention and control of CDI:

1-Control of risk factors. When we use antibiotics is important an appropriate antimicrobial stewardship. If was possible, we would have to stop antibiotic treatment in patients with CDI. In most cases, we cannot eliminate the antibiotic treatment so we should decrease the use of broad spectrum antibiotics for narrower spectrum ones. (63, 65, 67, 69, 185) Another factor is malnutrition of patients which is a predisposing factor in a long term care facility. (70)

2-Early diagnosis (high diagnostic suspicion). Axelrad et al.(113) studied the use of nursing admission (protocol) workflow to increase the rate of CDI testing. The intervention increased CDI testing for IBD inpatients with a relapse. We have to suspect in outpatient with a relapse-resistant to conventional treatment or with a relapse and risk factors for the infection. Moreover, in all hospitalized due to a relapse or worsen during the hospitalization.

3-Hygiene measures and prophylaxis CDI, to prevent spread by the medical staff to other patients and contamination with spores the hospital environment and surfaces and to avoid horizontal transmission. Patients usually acquire the organism from the hospital, no from their flora. Unfortunately, the spores

are difficult to eliminate from hospital wards, and some hospitals have experienced CD outbreaks that continued for years. Patient to patient spread may be a more important cause of increased CDI rates. (69) Secondary transmission among the patients was facilitated by the close rooms, shared bathrooms and living areas, and socialization with other patients. Moreover, it is possible to find environmental contamination in non-isolation rooms, in physicians and nurses work areas, and on portable equipment. Another research is needed to determine whether contamination in these areas could play a role in CD transmission.(88)

On the contrary, Daneman et al. (186) found that selected hospital prevention strategies were not associated with a statistically significant reduction in patients' risk of CDI. These strategies had limited effectiveness or were ineffectively implemented at least, during that study period.

It is important to recognize that CD outbreaks can occur in residential homes. Health- and social-care systems have to work closely together to assure the safety of people in their care.(98)

To sum up, most important methods of prevention are antibiotic stewardship, hand hygiene, isolation, and barrier methods in the hospital and long-term care facilities settings. The isolation is maintained up to 48 h after the enteric resolution. Patients in a separate ward, education of staff, and intensified environmental cleaning. (65, 185) Another control measures such as communication, education, reinforcement of infection control measures, optimization of diagnosis and treatment.(187)

Mayo Clinic researchers recommend practicing prevention, including:

- Wash hands with soap and water.
- Clean suspected contaminated surfaces with bleach-based solutions (1000 pm).
- Avoid contact with people with CDI.
- Take precautions if you are living with a person who has CDI or works in a healthcare setting where might be exposed to patients with CDI.

Finally, we would like to highlight the importance of a continuous CDI surveillance in the hospitals, especially when a risk group is exposed as IBD patients. An active surveillance and prevention campaign such as “wash hand campaigns” are necessary for all hospitals.

Chapter 2

3. Chapter 2: Study

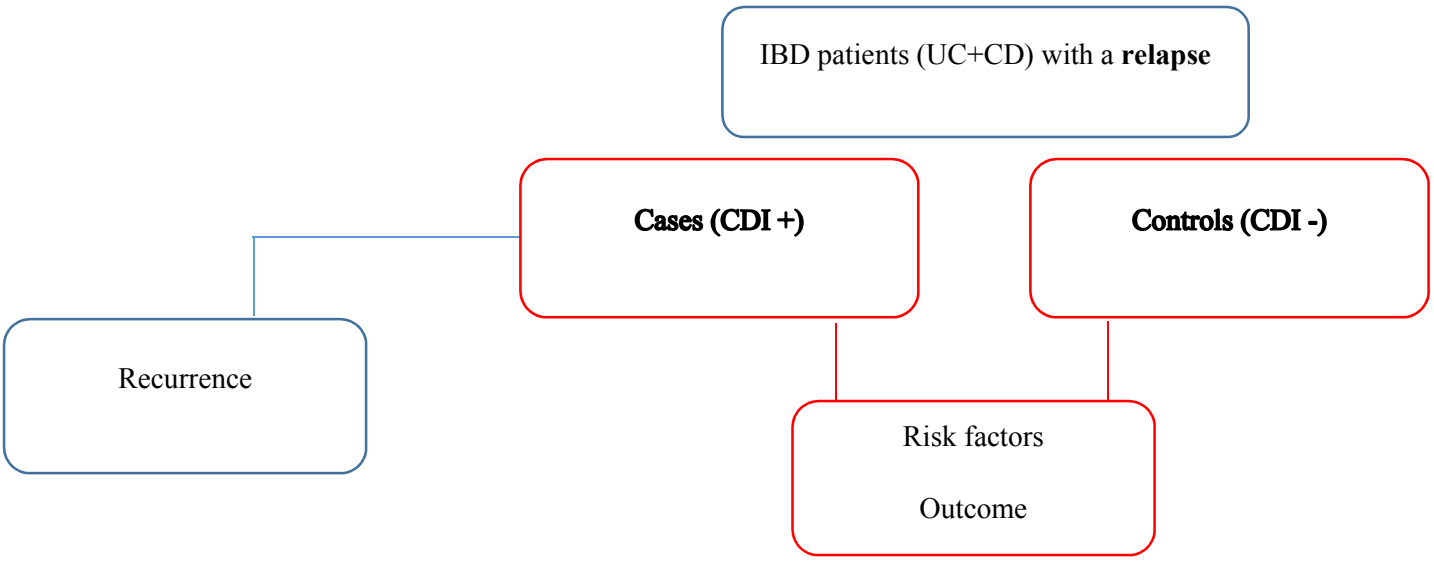
3.1 Aims

The purposes of this study are:

- Describe CDI episodes and IBD characteristic to find risk factors for CDI in IBD patients with a relapse.
- Analyse the recurrence of CDI in IBD patients with a relapse
- Investigate the influence of CDI on IBD outcome and
- Establish a patient profile at risk for CDI in our area

3.2 Methodological section

Graphic design study



Patients

We performed a retrospective case-control study in IBD with a relapse, including adult patients (aged > 14 years). Study was set in the Departments of Digestive Disease and Microbiology of a single tertiary teaching hospital in Las Palmas of Gran Canaria (Spain) during the period from June 2007 to June 2015.

Inclusion criteria: cases were defined as IBD patient with a relapse (diarrhoea with liquid stools), with positive CD in stools samples. Controls were also IBD patients with a relapse but with negative CD in stools samples.

Recurrence: a return of signs and symptoms of CDI after a period of wellness with a positive stool test for CD in any evaluation period. *Recurrence*: When CDI re-occur <8 weeks after the onset of a previous episode, with symptoms from the previous episode resolved after completion of initial treatment. *Re-infection*: CDI re-occur ≥8 weeks after the onset of a previous episode with symptoms from the previous episode resolved after completion of initial treatment.

Risk factors were considered: to take antibiotics and PPIs 3 months before episodes and hospitalization 3 months before episodes.

Outcome was considered: therapeutic escalation and hospitalization 6 months after episodes and surgery (colectomy) 1 year after episodes

Episode of CDI: a clinical picture compatible with CDI (diarrhoea: three or more loose stools per day for two or more days) and microbiological evidence of free toxins and the presence of CD in stools, without reasonable evidence of another cause of diarrhoea.

Severe CDI: Severe or life-threatening CDI is defined as an episode of CDI with (one or more specific signs and symptoms of) severe colitis or a complicated course of disease, with significant systemic toxin effects and shock, resulting in need for ICU admission, colectomy or death. One or more of the following unfavourable prognostic factors can be present without evidence of another cause: marked leucocytosis (> 15 10^9/ L), decreased blood albumin (< 3 gr/L) and rise in serum creatinine level (>1.5 times the premorbid level).

Severe IBD: mild, moderate or severe based on subjective physician global assessment. We could not evaluate activity indexes because our study was retrospective one and we did not have all data for calculating them.

An exclusion criteria for controls was CDI known previously.

Methods

Cases and controls were identified from electronic database of the Laboratory of Microbiology of our hospital. Controls were randomized with simple random sampling performed with Excel 2010 and matched 1:1 for period of time with the cases. CDI was diagnosed by stool samples tested for CD toxins A and B by an ELISA assay from 2007 to December, 2012 and by two steps test (GDH and toxins A and B) and PCR from January, 2013.

The following data were collected from patients' medical records: epidemiological risk factors, clinical data, IBD characteristics including location, therapy, 3 month prior surgeries, hospital admissions and laboratory information (including faecal calprotectin). In addition, we studied the recurrence and outcome: escalation therapeutic and hospitalizations 6 months after episodes, surgery (colectomy) 1 year after episodes, complications and deaths

Analyse of data was performed with SPSS program: statistical comparisons were performed with the Student's t (quantitative variables) and X2 tests (qualitative variables) and when it was necessary Fisher and U-Man Witney´s test (OR with 95% CI). Logistic regression analysis was performed with variables that were significant in univariate analysis to identify risk factors for CDI

Chapter 3

4. Chapter 3: Results

From June 2007 to June 2015, we analysed 131 CDI episodes in IBD patients with a relapse (case group) comparing with IBD patients with a relapse but without infection (control group). Moreover, we studied the recurrent CDI episodes.

We are going to show you the results of our study with the following outline:

- Firstly, we would like to give you a general vision of the distribution of CDI (positive results); a comparison of positive results in IBD patients with positive results in non-IBD patients in the same period of time and the proportion between request samples within the total sample and final positive results.
- Secondly, we are going to see how long it takes to diagnose these patients in our hospital.
- Thirdly, we are going to explain the results of case-control study including risk factors and outcome.
- And finally, we are going to comment our results in the study of recurrent episodes.

4.1 Distribution of CDI episodes

In our tertiary centre, we do not have found an increase in the number of CDI episodes in both, non-IBD and IBD patients during the period of the study. Moreover, the number of positives CDI episodes tends to maintain or decrease but in no case has increased last 8 years. We realized that proportion of positive results was low compared with the number of request samples.

4.1.1 Positive episodes distribution during the period of our study

Distribution of positive samples from June 2007 to June 2015. It can be seen as the number of positives episodes have been decreasing during the period of the study. Thus, from January to June 2015, we do not have had any case. (Figure 1)

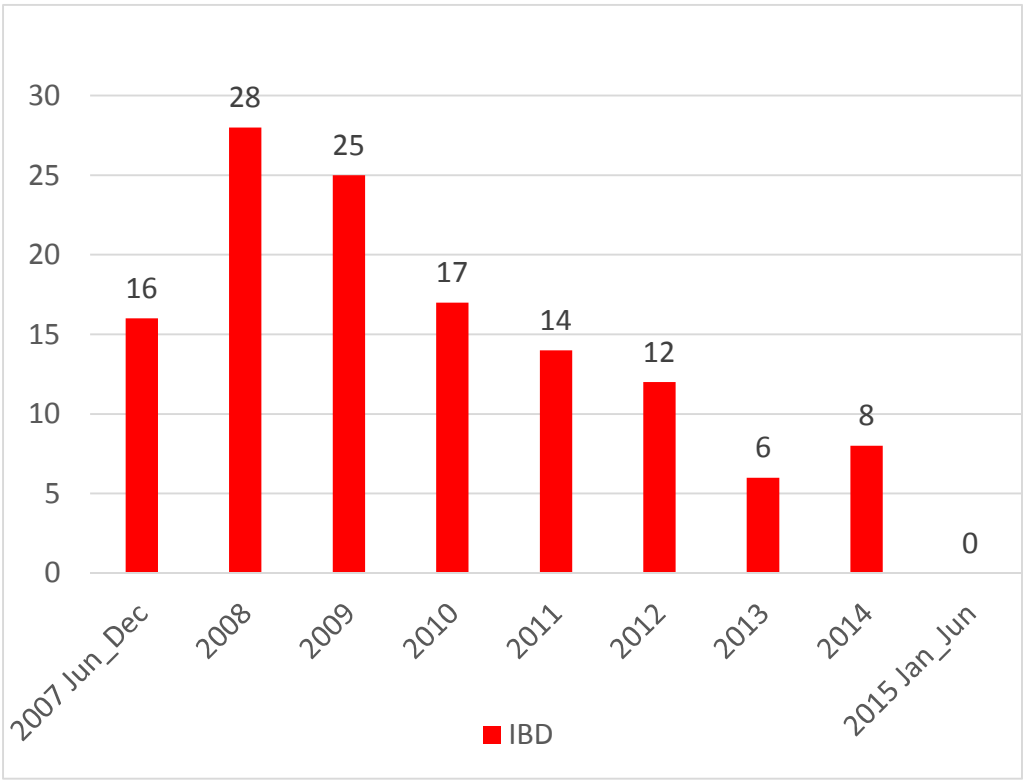


Figure 1. Distribution of positive samples from June 2007 to June 2015.

4.1.2 Positive episodes distribution comparing with general population

Positive episodes distribution in IBD patients compared with positive episodes in non-IBD population from June 2007 to June 2015. The distribution of CDI in general population has not been increasing during the last 6 years, but at the same time it has been maintained in similar proportions in contrast to IBD population. (Figure 2)

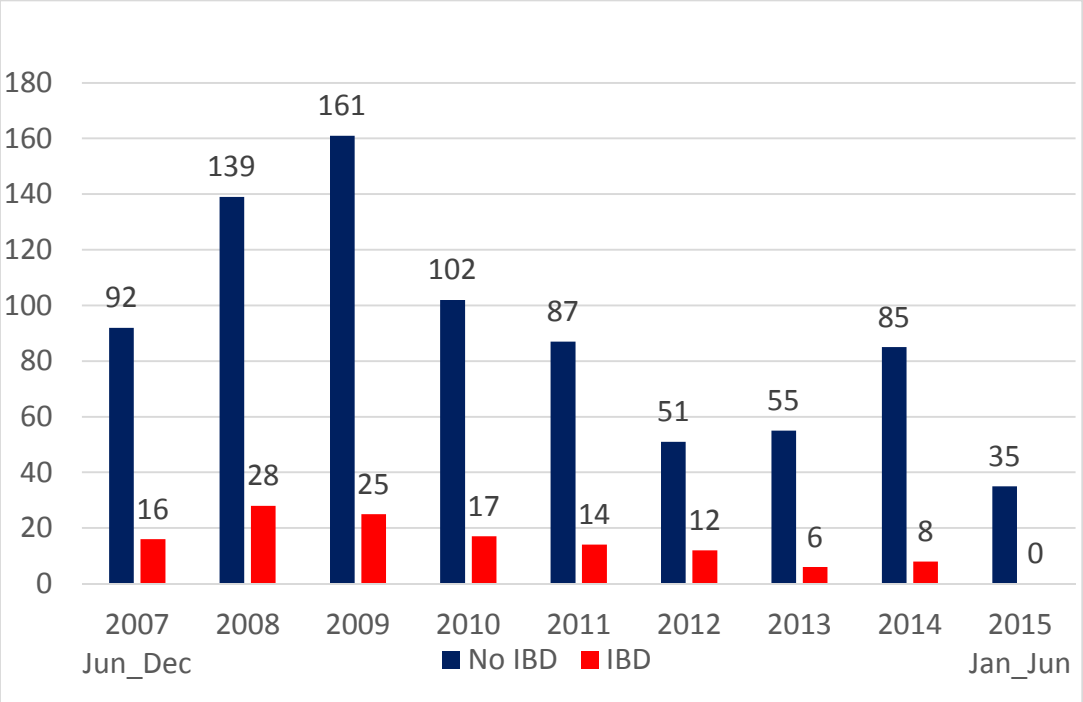


Figure 2. Positive episodes distribution in IBD patients compared with positive episodes in non-IBD population from June 2007 to June 2015.

4.1.3 Total requested samples in IBD patients compared with positive final results

Total requested samples in IBD patients compared with positive final results. The number of requested samples was higher than the number of positives results in a very striking way. We found a low proportion of positive results compared with a large number of requests. (Figure 3)

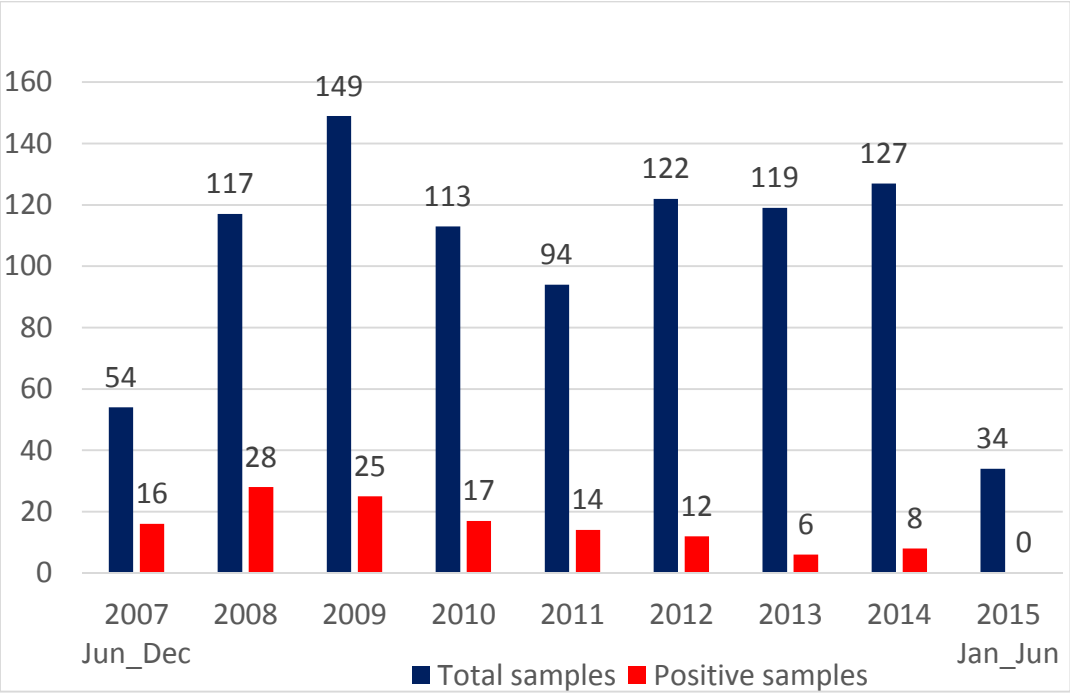


Figure 3: Total requested samples in IBD patients compared with positive final results.

4.2 Time it took to diagnose our patients

The time it takes for the positive results to come out from Microbiology department was 4.22±6.281 days, the time it takes from positive result release to doctor´s appointment was 7.59±6.223 days and time it takes from first doctor´s appointment to result delivery to the patient was 15.58±8.265 days.

In our department, CDI took an average of 14 days to diagnose, but the positive result was coming out from the Microbiology Department in an average time of 2 days. Therefore, there is an untapped space of about 10-12 days in which it would be possible to know the samples results. (Table 1)

Table 1: Days to diagnose CDI in outpatients.

	Time it takes for the positive result to come out (Microbiology Department)	**Time it takes from positive result release to doctor´s appointment**	**Time it takes from first doctor´s appointment to results delivery to patient**
Mean (days)	4.22±6.281	7.59±6.223	15.58±8.265
Median	2.00	7.00	14.00
Mode	2	7	14
Minimum-maximum	1-53	0-30	4-42

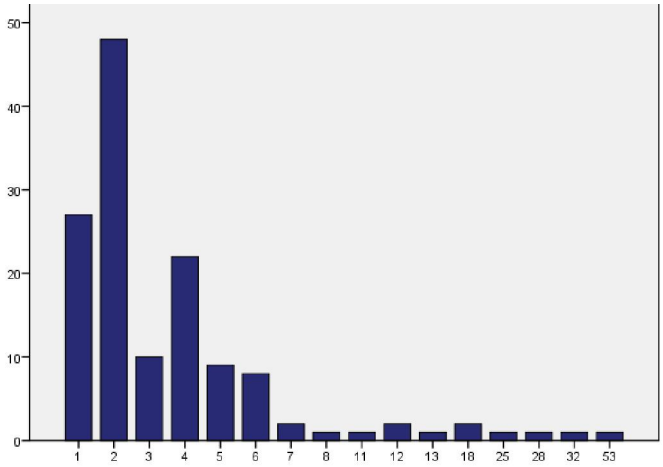


Figure 4: Days that it takes for results come out from the Microbiology Department.

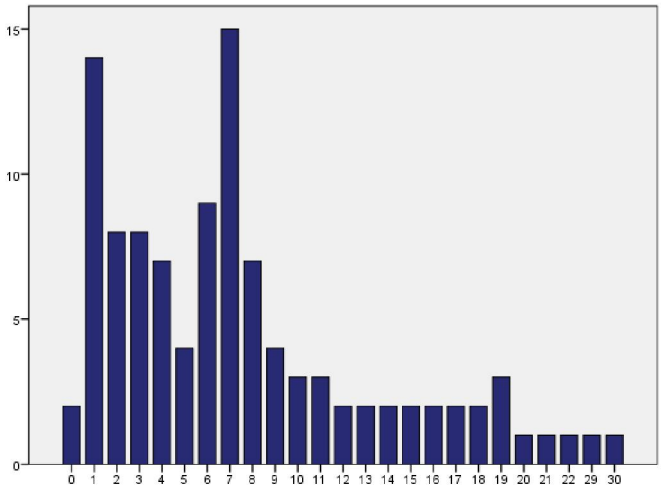


Figure 5: Distribution of days since the positive result was available until IBD patients visited the doctor.

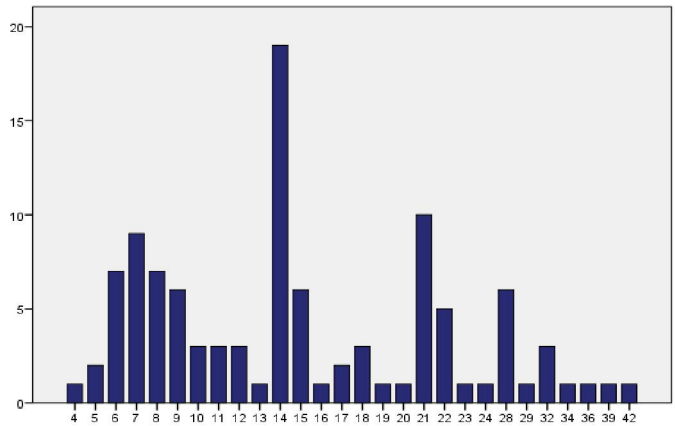


Figure 6: Days since first doctor´s appointment until the patients go back again to know the results.

4.3 Case-control study

From June 2007 to June 2015, we analysed 131 Clostridium difficile infection (CDI) episodes in IBD patients with a relapse (case group) compared with IBD patient with a relapse but without CDI (control group).

4.3.1 General characteristics of episodes

The results were the following in cases and controls respectively (see table below). We did not find significant differences between both groups in mean age and gender. CDI episodes presented in less than 10% in patients >65 years old. In Autumn-Winter occurred: 28% (37) and 18% (19) of episodes. There was a smoking history: 20% (27) and 18% (19); in case group CD 67% (18) (OR 2.47; CI 95% 1.63-3.74, p=0.00) and in control group CD 89% (17) (OR 1.60; CI 95% 1.26-2.04, p=0.00). We did not have CDI episodes in patients with previous colectomy (neither in the control group). Average ages were predominant in both groups at IBD diagnosis: ≤16: 6% (8) and 11% (12); 17-40: 71% (93) and 65% (70); >40: 23% (30) and 22% (24).

Table. General characteristics of episodes

	Case	**Controls**	**p value**
Mean age	38.11±14.759	38.46±15.547	NS
>65	10 (8%)	6 (6%)	NS
Gender			
Female	76 (56%)	57 (53%)	NS
Male	55 (42%)	50 (47%)	
Season			
Autumn-Winter	37 (28%)	19 (18%)	p=0.05
Spring-Summer	94 (72%)	88 (82%)	
Smoking history	27 (20%)	19 (17.7%)	NS
UC	9 (33%)	2 (11%)	
CD	18 (67%) p=0.00	17 (89%) p=0.00	
Previous surgery (colectomy)	11 (8.3%)	17 (15.8%)	NS

Age at IBD diagnosis			NS
≤16	8 (6%)	12 (11%)	
17-40	93 (71%)	70 (65%)	
>40	30 (23%)	24 (22%)	

Distribution of IBD in case and control group was respectively: UC 65% (85) and 38% (41); CD 35% (46) and 62% (66) (OR 1.64; CI 95% 1.27-2.11, p=0.00).

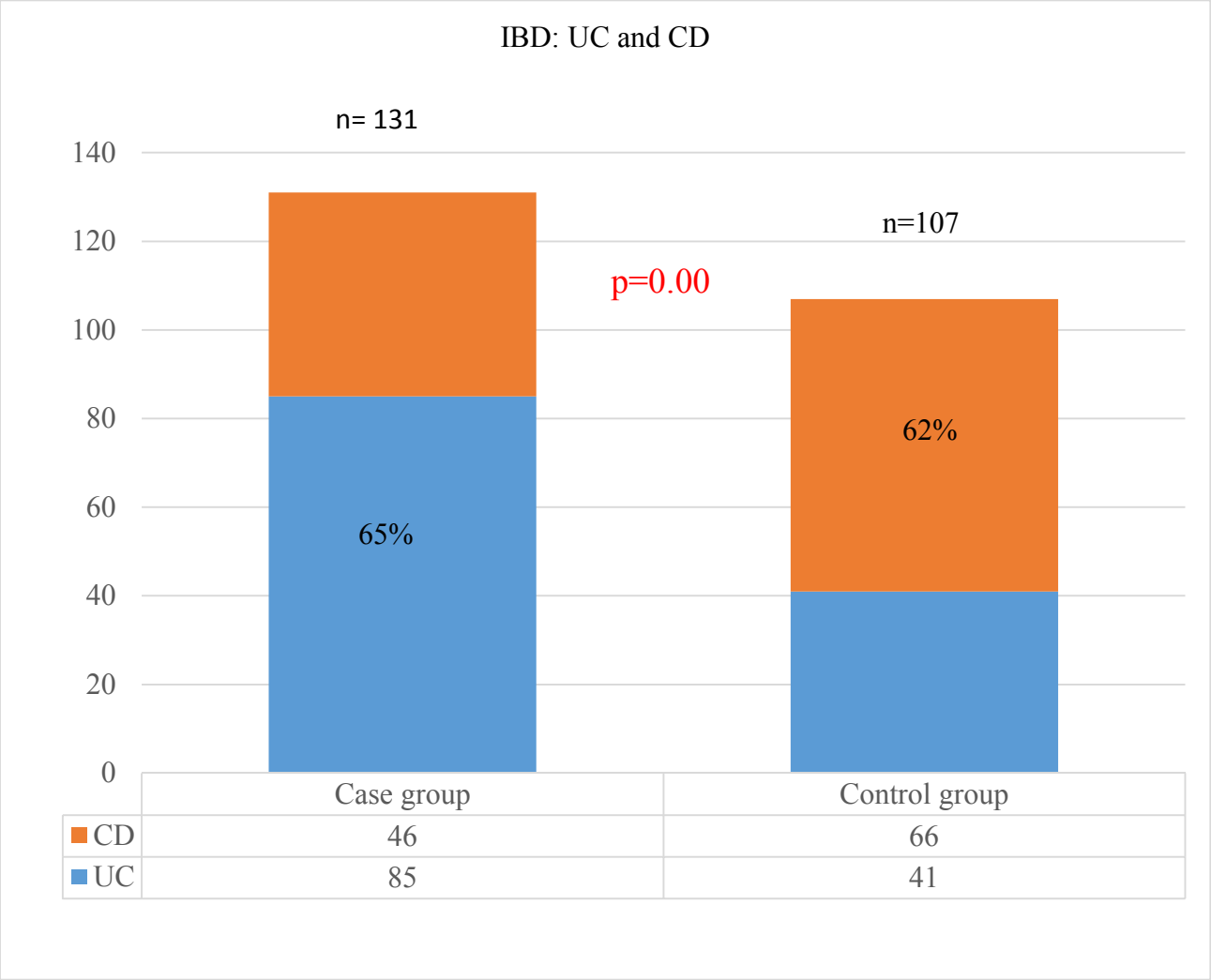


Figure. IBD distribution (UC and CD)

Ulcerative colitis with rectal location is more frequent in case group compared with controls (OR 1.37; CI 95% 1.09-1.71, p=0.04). We did not find differences between both groups in location (Proctitis+ left) compared with extensive.

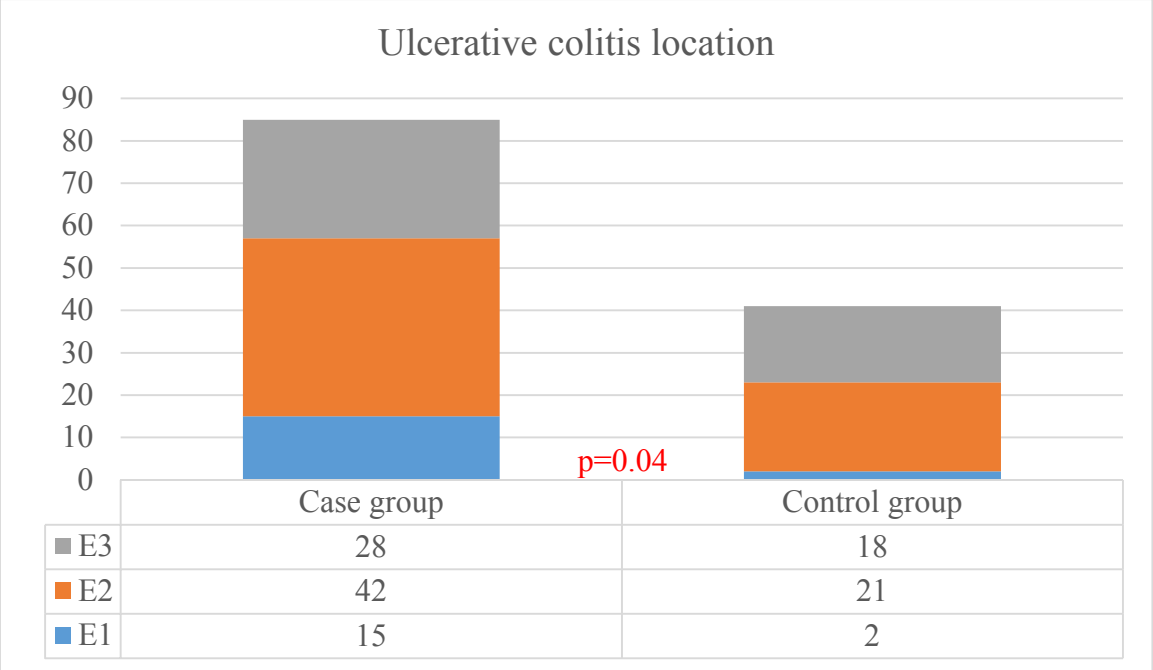


Figure. Ulcerative colitis location: E1: proctitis; E2: left; E3: extensive

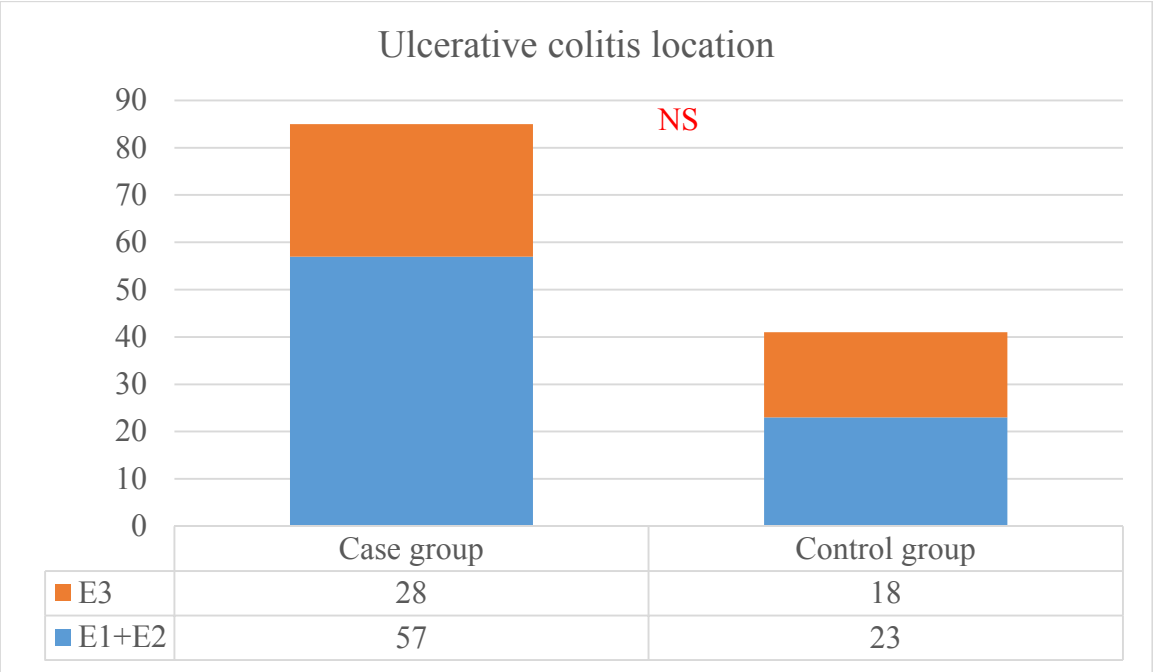


Figure. Ulcerative colitis location: Proctitis+ left and extensive.

Crohn´s disease location in case and control group respectively: colonic involvement (L2 and L3) is more frequent in cases: L2 (colonic): 39% (18) and 30% (20); L3 (ileo-colonic): 28% (13) and 15% (10) (OR 1.69; CI 95% 1.03-2.76, p=0.02). However, ileal location (L1) is more frequent in controls: 33% (15) and 53% (35) (OR 1.37; CI 95% 1.01-1.86, p=0.03). Perianal location is more frequent in cases than controls: 20% (9) and 8% (5) (OR 1.72; CI 95% 1.07-2.74, p=0.05).

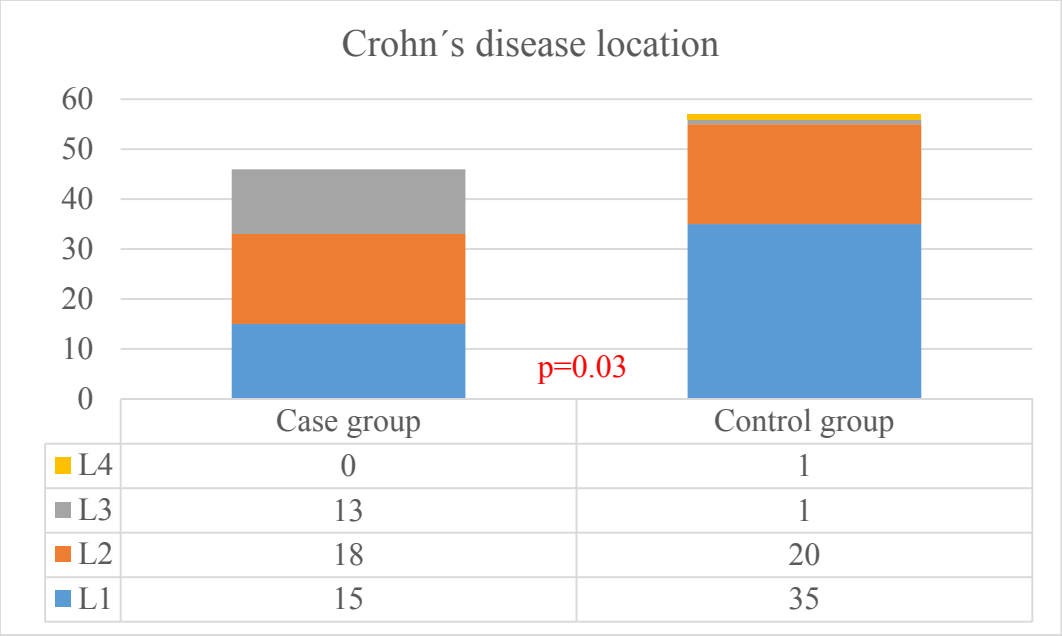


Figure. Crohn´s disease location: L1 (ileal), L2 (colonic), L3 (ileo-colonic)

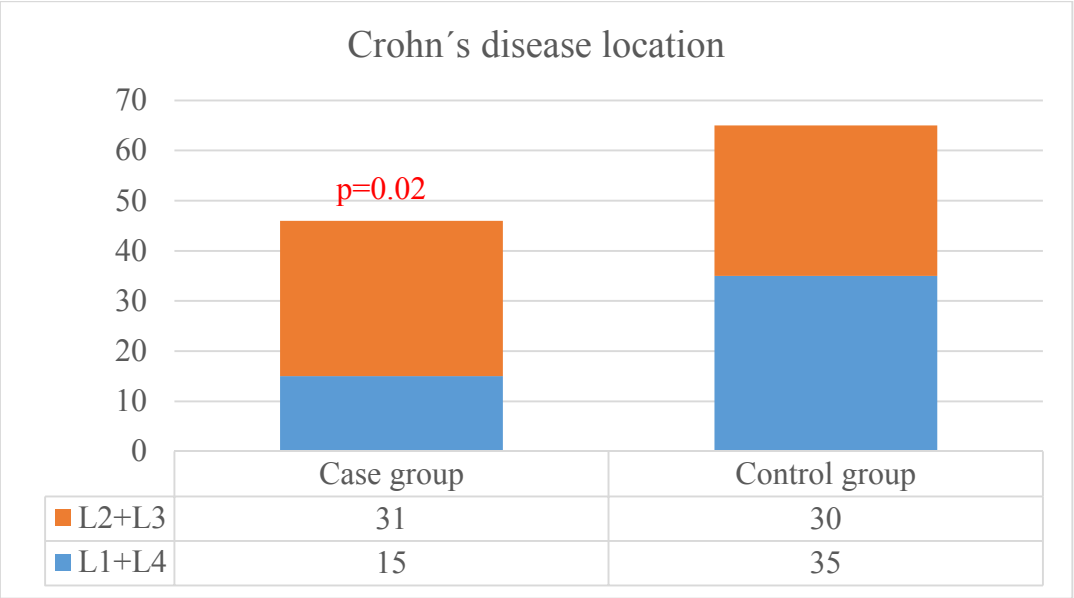


Figure. Crohn´s disease location: L1+L4 and L2+L3 (colonic involvement).

Crohn´s disease, there were not differences between case and control group between non-stricturing, non-penetrating (B1), stricturing (B2) and penetrating (B3) behaviors. However, non-stricturing, non-penetrating behavior was more frequent in cases compared with estricturing (OR 2.18; CI 95% 1.11-4.30, p=0.00). We found more episodes with abscesses in cases than in controls (p=0.06).

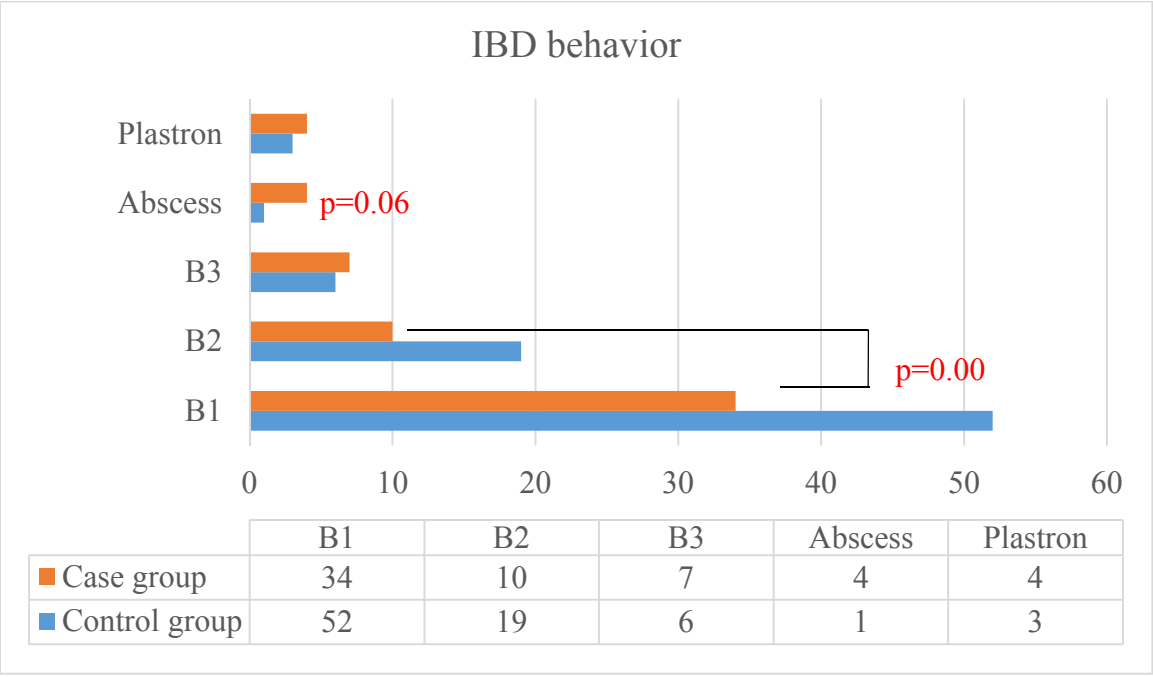


Figure. IBD behavior

Overall, we did not find differences in evolution time from IBD diagnosis until episodes. However, we realized that the behavior in both groups was different. Thus, the probability of relapse with/without infection along the time was different comparing cases-controls and UC-CD.

	Cases	**Controls**	**p value**
Evolution time of IBD (years)	4.41±4.702	4.01±4.347	NS
UC	4.48±4.264	3.37±4-048	
CD	5.22±6.077	4.41±4.506	

CDI was frequent during the first 3 -7 years after IBD diagnosis. A quarter of the episodes occurred in the first year after IBD diagnosis (50% and 70% respectively). Thus, IBD patients with CDI had more relapses the first 7 years after IBD diagnosis (with more concentration of cases in the first year). (See pictures below)

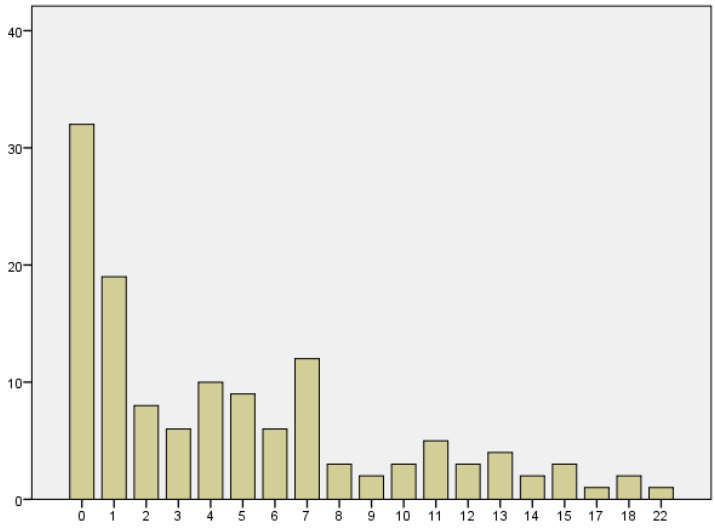


Figure. Distribution of episodes from IBD diagnosis to relapses (cases)

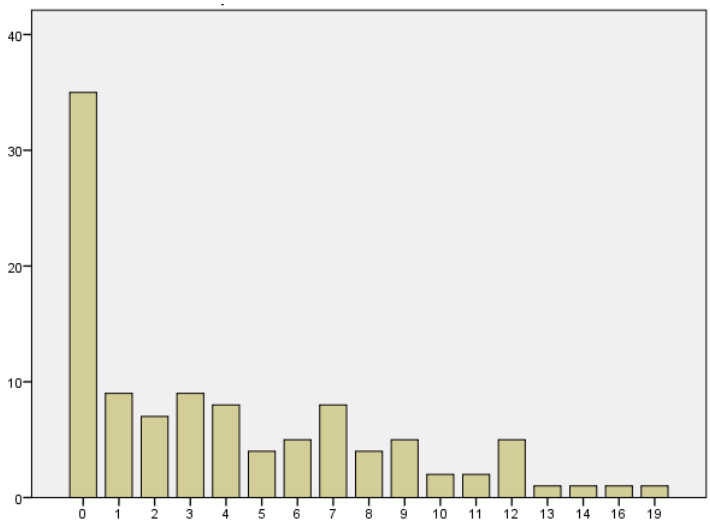


Figure: Distribution of episodes from IBD diagnosis to relapses without CDI (controls)

UC episodes with CDI had more relapses the first 7 years and earlier compared with CD

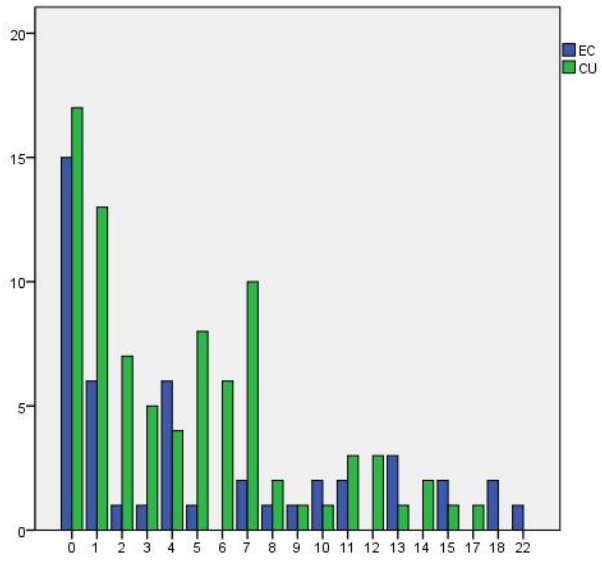


Figure. Cases (UC and CD)

CD and UC episodes without CDI had more relapses the first year and after CD had more relapses and the number of UC episodes stabilized.

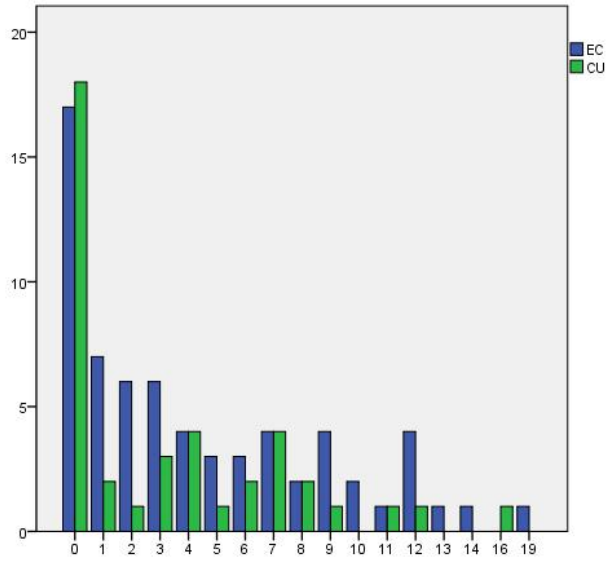
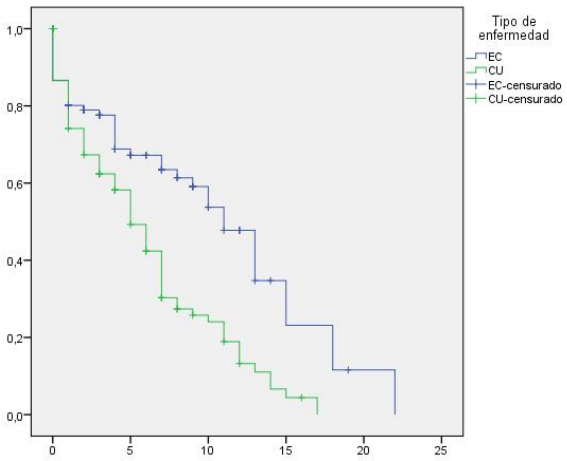


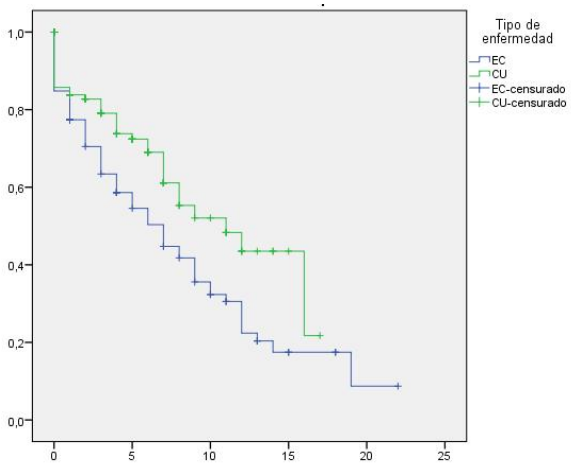
Figure. Controls (CD and UC)

The behavior of UC and CD with and without CDI was different: CDI makes the behavior of ulcerative colitis similar to the behavior of Crohn´s disease without CDI. Moreover, UC with CDI seems to have a different behavior as compared with UC without infection. UC with the infection has more probability of relapse and earlier than UC without infection, where the evolution only depends on its natural evolution. In contrast, in CD with the infection the relapses were less frequent when compared with CD without infection, in which the relapse depends on the natural evolution of the disease, without an external element triggering the relapse. (See figures)

UC episodes with CDI (cases), had more probability of relapse the first 7 years, with a different behavior compared with CD.



In control group: probability of relapse without infection in UC and CD from IBD diagnosis (years)



UC with CDI had more likely to relapse compared with UC without infection.

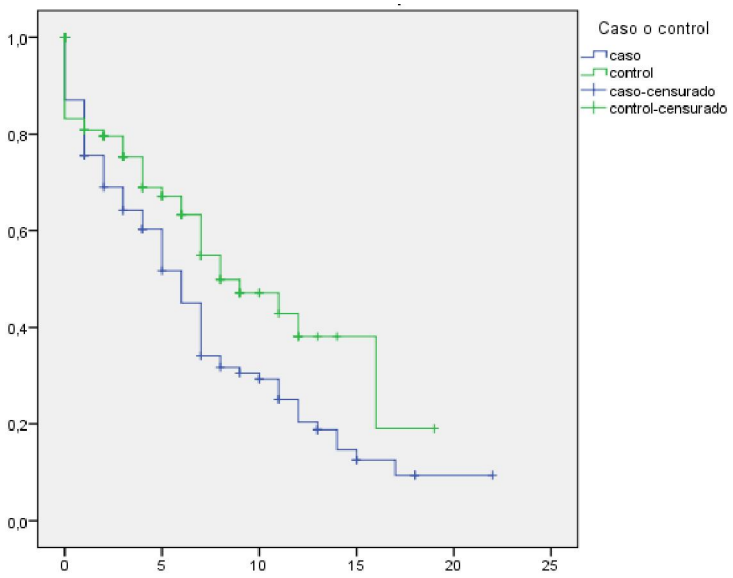


Figure. UC probability of relapse with/without infection from IBD diagnosis (years)

Relapse in CD episodes was earlier without infection (when the relapse was by IBD per se). When the relapse was because of CDI the behavior was better (with an external cause).

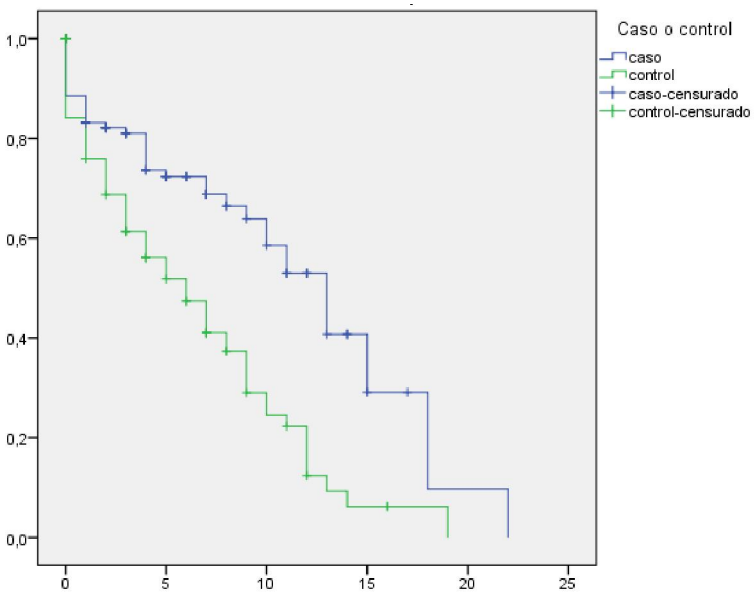
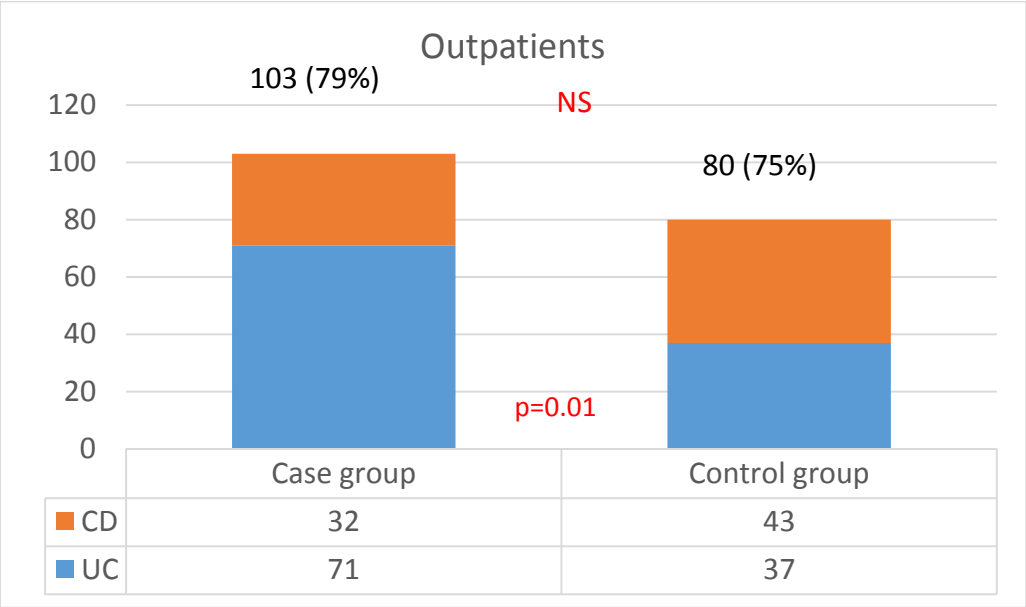
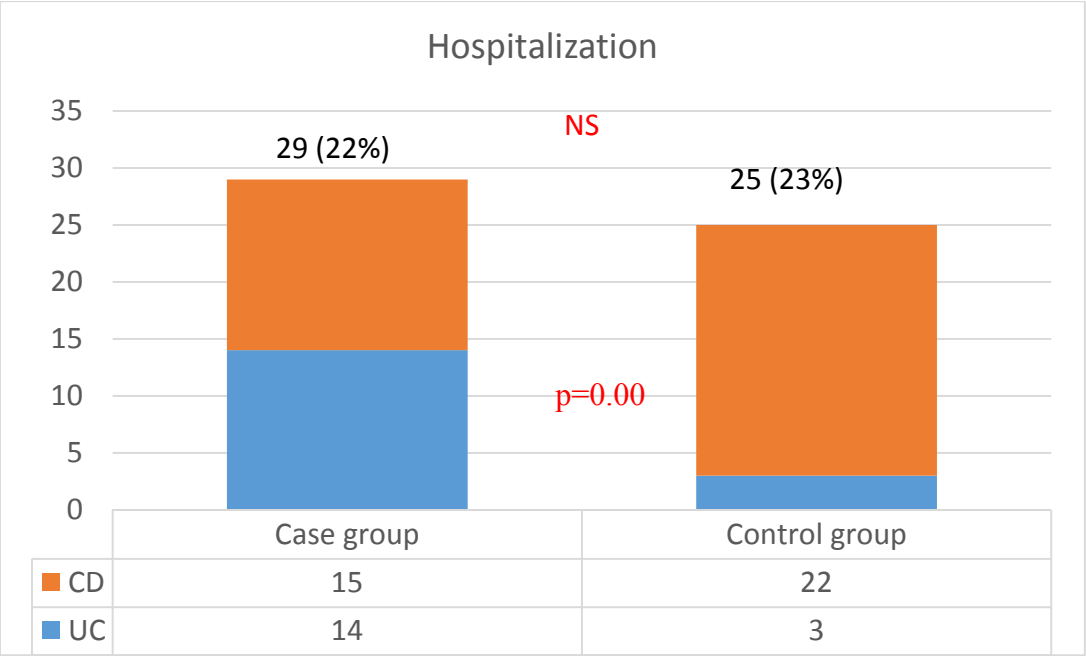


Figure. CD probability of relapse with/without infection

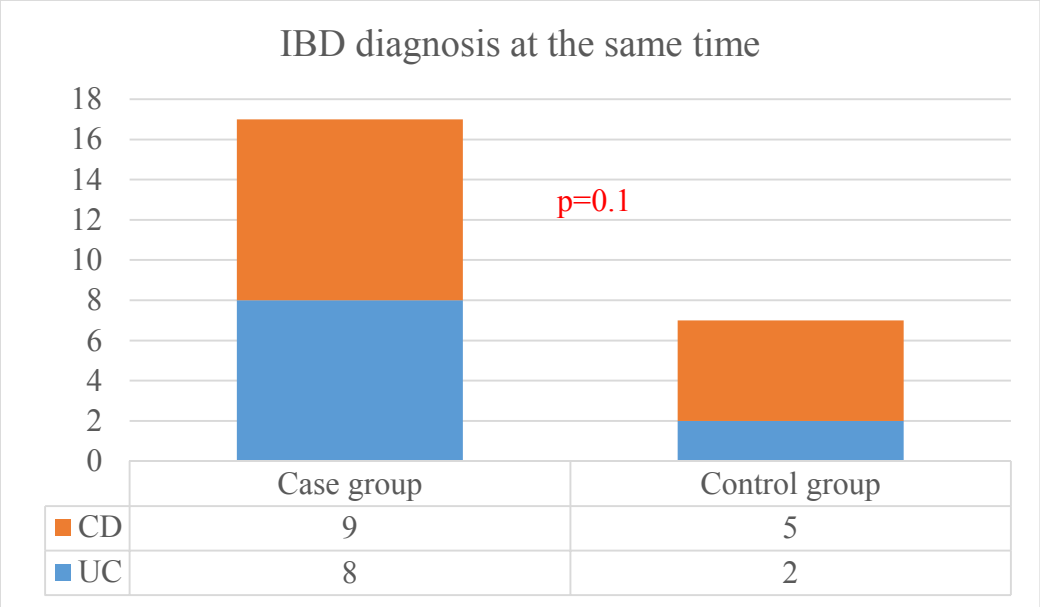
Samples were requested as outpatient in more than 70% of episodes in cases and controls: 78% (103) and 75% (80) respectively. When we analyzed separately UC and CD in case and control group we found in cases 69% (71) UC and in controls 54% (43) CD (p=0.01).

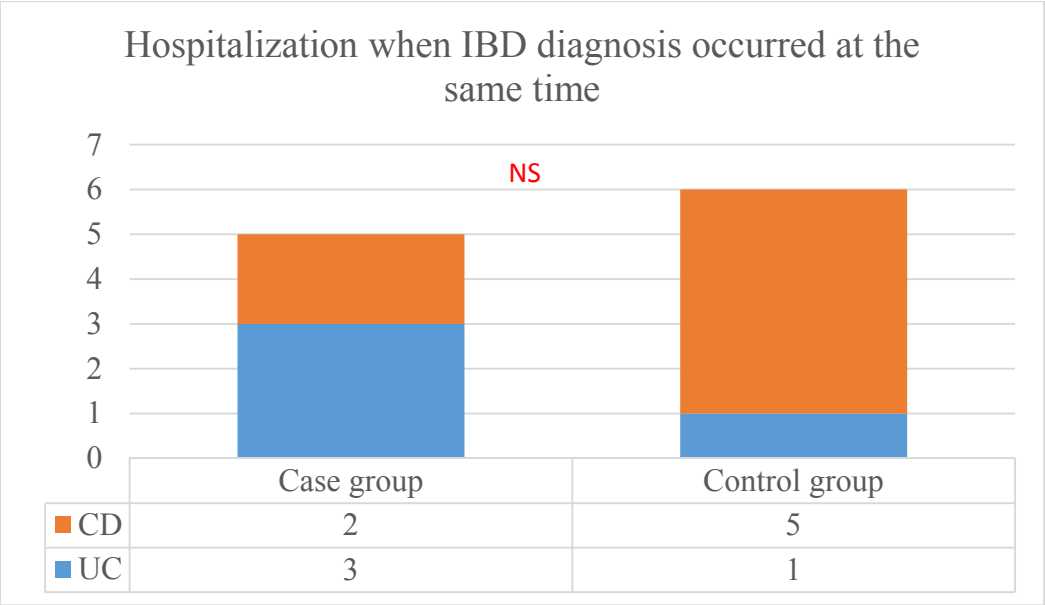


Episodes with hospitalizations in case and control group were similar: 22% (29) and 23% (25) respectively. Hospitalization in controls was higher in CD 88% (22) compared with UC 12% (3) (OR 1.64; CI 95% 1.28-2.10, p=0.00) but in cases was similar. Thus, CDI. Average stay in cases and controls were 12.03±9.697 and 14.22±13.497 respectively, without differences.

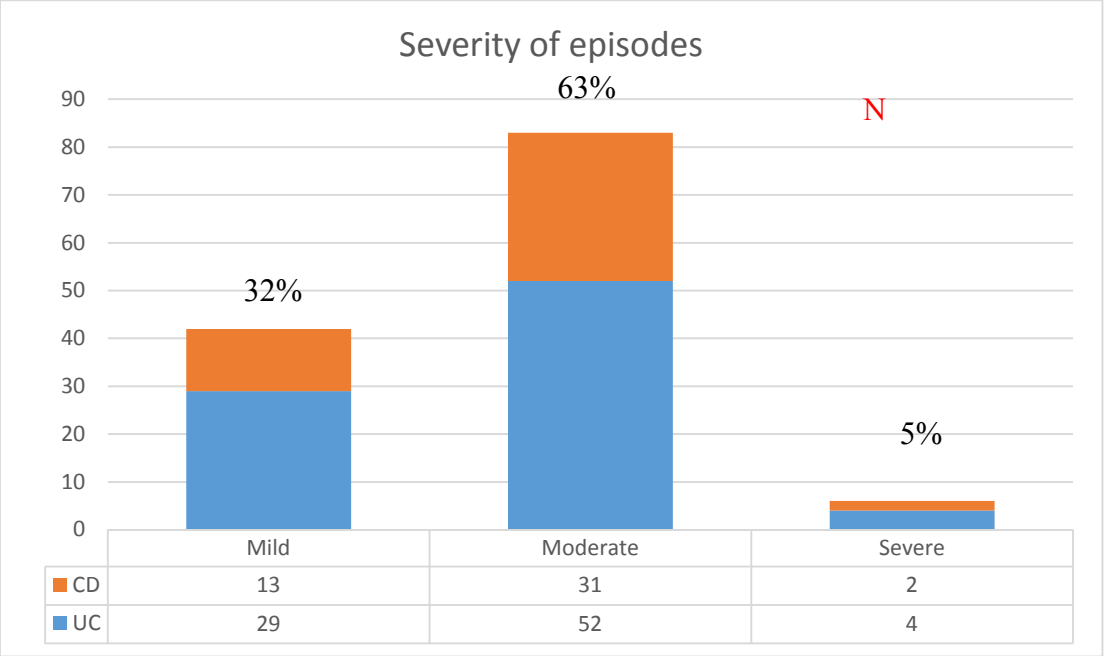


Episodes at IBD diagnosis and relapse with/without CDI occurred at the same time comparing case and control group: 13% (17) and 7% (7) (p=0.1). 13% of cases had an IBD diagnosis and CDI at the same time, without differences between UC and CD (see figure below). IBD diagnosis and relapse with/without CDI at the same time and hospitalization in cases and controls were: 4% (5) and 6% (6) respectively (see figure below)

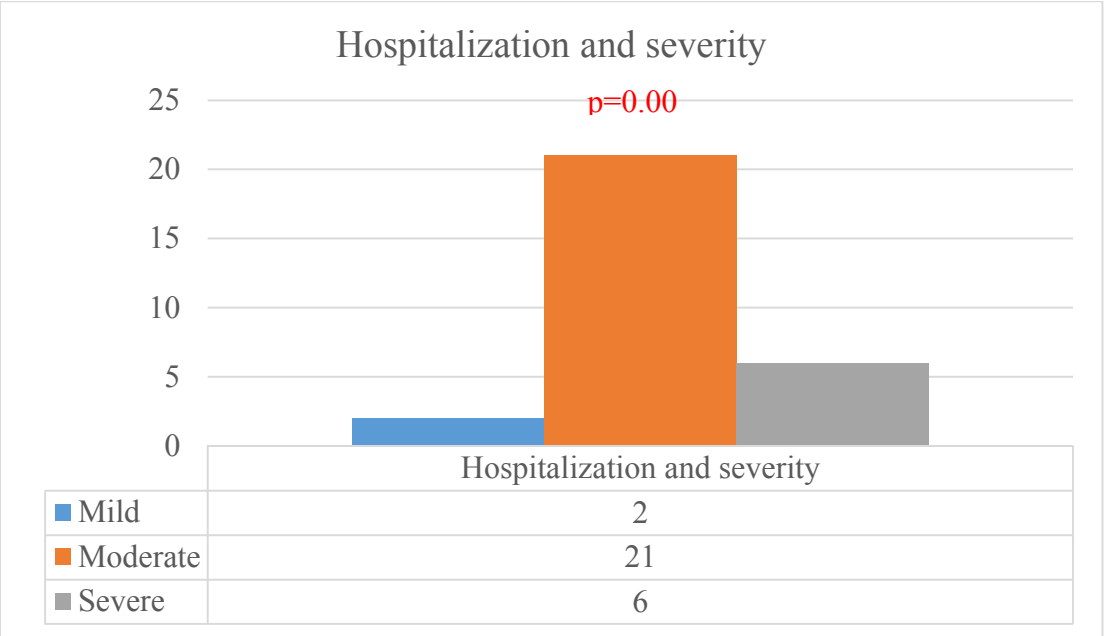




The severity of episodes in case group was more frequent mild-moderate: 32% were mild ones. More than 50% had moderate severity, and only 5% of episodes were severe. There were not differences between UC and CD. We did not find differences compared with the control group. When applied criteria of severe CDI, we found most episodes were not severe ones.

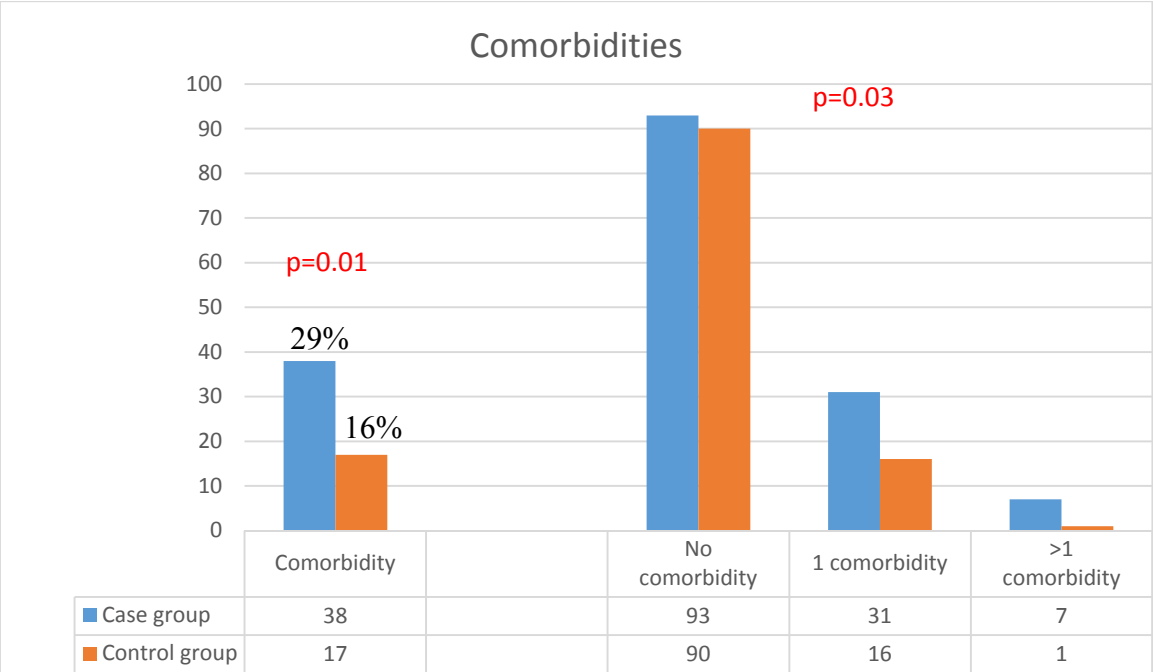


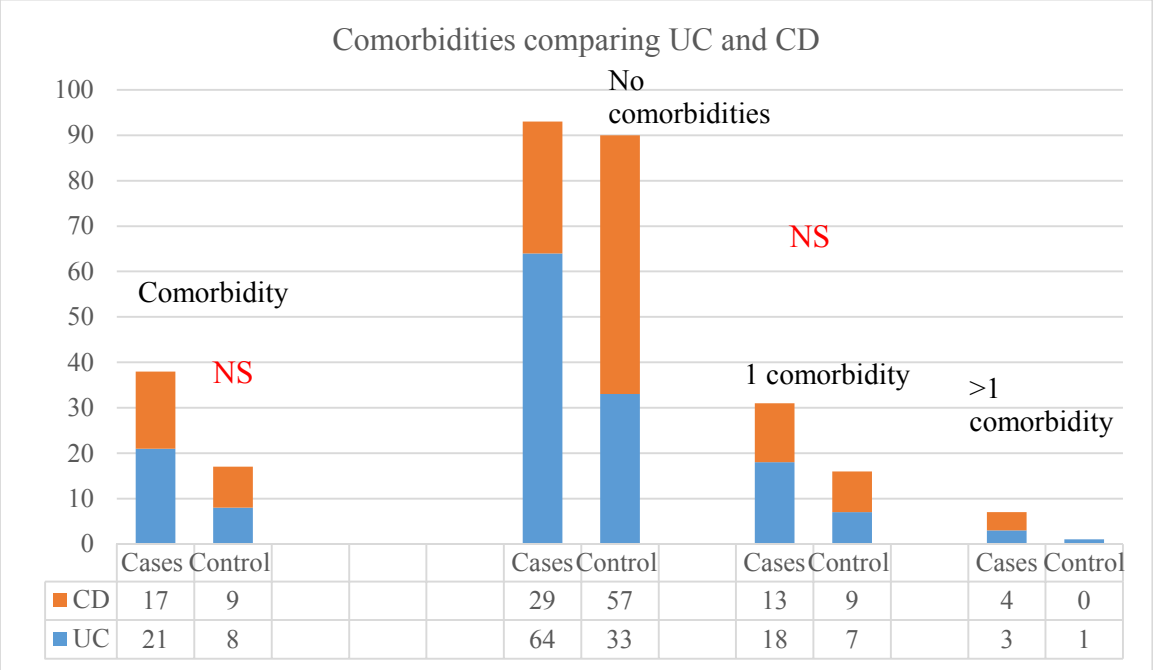
Severity and hospitalization in case group: mild; 7% (2), moderate; 72% (21), severe; 21% (6) but without differences with control group.



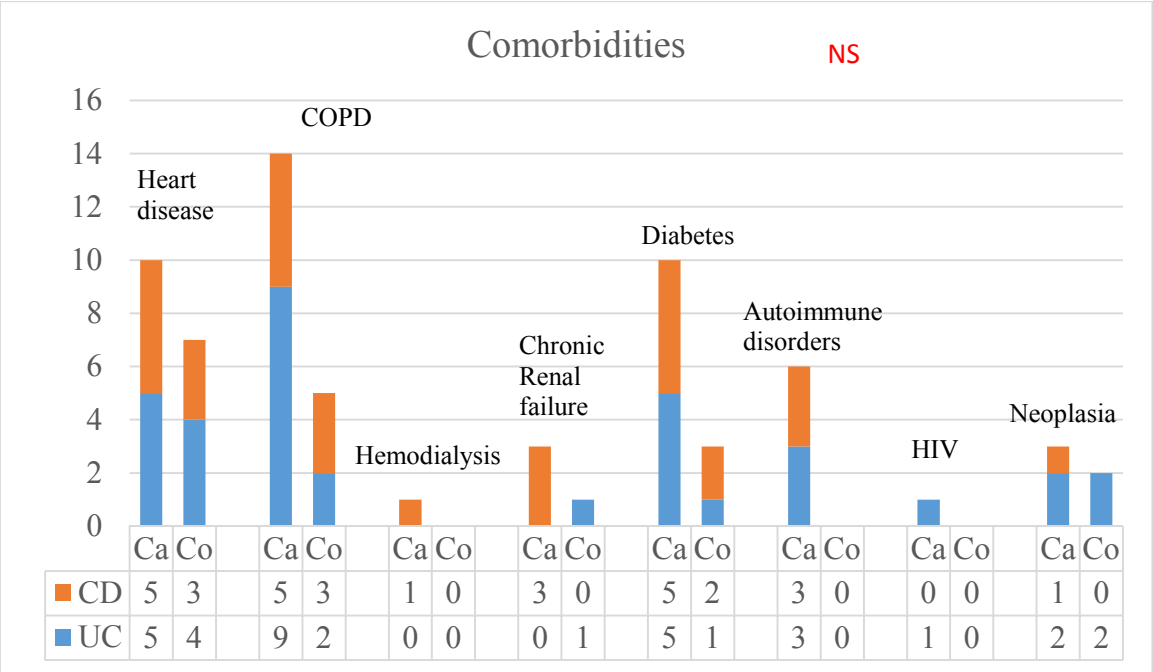
Comorbidity in episodes: CDI patients presented more comorbidity than control group: 38 (29%) and 17 (16%) (OR 1.36; CI 95% p=0.01). Numbers of comorbidities in cases and control: 0; 71% (93) and 84% (90), 1; 24% (31) and 34% (16), >1; 7 (5%) and 1 (0.9%) (p=0.03). There were no differences between UC and CD in cases and controls. (See figure below).

In cases group: comorbidity and severity in cases group: mild 29% (12), moderate 31% (26), severe 0% (0) (there is not comorbidity in severe episodes). Number of comorbidities and severity: 1comorbitiy and mild episode 24% (10), moderate 25% (21) and severe 0% (0) and>1 comorbidity and mild 5% (2), moderate 6% (5) severe 0% (0) (no differences with control group)

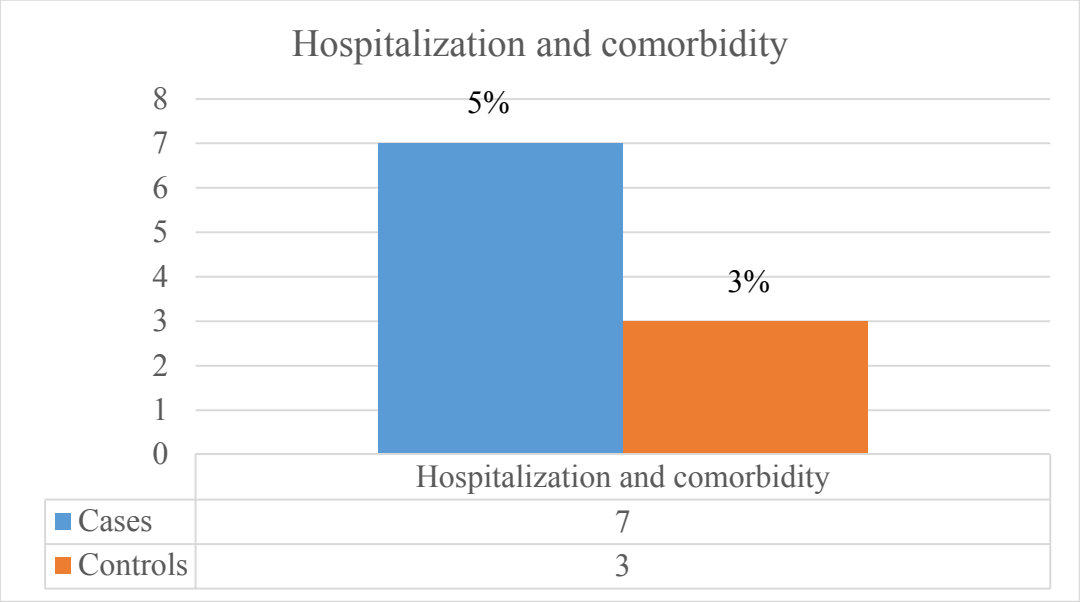




We found a higher proportion of chronic obstructive pulmonary disease (COPD), heart disease and diabetes in cases but without differences with controls.


Comorbidities
NS
16
14
12
10
8
6
4
2
0
Heart disease
COPD
Hemodialysis
Chronic Renal failure
Diabetes
Autoimmune disorders
HIV
Neoplasia
Ca Co Ca Co Ca Co Ca Co Ca Co Ca Co Ca Co Ca Co
CD 5 3 5 3 1 0 3 0 5 2 3 0 0 0 1 0
UC 5 4 9 2 0 0 0 1 5 1 3 0 1 0 2 2

Hospitalization in patients with comorbidities had a tendency to be higher in case group but without differences between both groups.



4.3.2 Laboratory parameters

We have not found statistic differences between cases and controls in inflammatory parameters such as CRP, ESR, ferritin, platelets levels and faecal calprotectin. However, calprotectin levels have a tendency to be higher in cases (p=0.06) (but we had a small samples sizes because we did not have samples in all episodes). We have not found differences in albumin levels between cases and controls. We have not found differences in haemoglobin, ferritin and Fe levels between cases and controls. Creatinine levels were normal in both groups without differences. (Neither if we compare ulcerative colitis and Crohn´s disease between them in both groups)

	Cases	**Controls**	**p value**
Faecal calprotectin	N=34	N=26	p=0.06
Mean	992.47	464.96	
Standard deviation	1558.400	396.558	
CRP	N=83	N=75	NS
Mean	2.79	3.00	
Standard deviation	4.77	5.09	

ESR	N=86	N=73	NS
Mean	26.40	28.97	
Standard deviation	20.63	31.40	
Albumin	N=53	N=37	NS
Mean	3.58	3.40	
Standard deviation	0.66	0.76	
Hemoglobin	N=96	N=78	NS
Mean	12.58	12.53	
Standard deviation	1.96685	1.99902	
Ferritin	N=67	N=67	NS
Mean	59.89	87.82	
Standard deviation	105.63	146.94	
Iron	N=53	N=60	NS
Mean	52.54	54.50	
Standard deviation	26.101	33.95	
Platelets	N=96	N=78	NS
Mean	350,750.92	333,243.58	
Standard deviation	140,265.104	132,638.573	
Leucocytes	N=96	N=78	
Mean	9,590.63	10,138.46	NS
Standard deviation	4,420.552	9,691.169	

Results of bivariate correlations analysis in cases:

We did not find significant positive correlations with $p<0.05$. Significant positive correlations were observed between ($p<0.01$):albumin and haemoglobin ($r=0.6$), ESR and CRP ($r= 0.4$), platelets and CRP ($r= 0.3$), ESR and platelets ($r=0.5$). Significant negative correlations were observed between ($p<0.05$): CRP and haemoglobin ($r= -0.2$), ESR and albumin ($r= -0.2$). Significant negative correlations were observed between ($p<0.01$):ESR and haemoglobin, albumin and CRP ($r= -0.4$)

Correlaciones

		Hemoglobina	PCR	VSG	Plaquetas	Albumina	Calprotectina fecal
Hemoglobina	Correlación de Pearson	1	-,233*	-,387**	-,196	,603**	-,009
	Sig. (bilateral)		,034	,000	,055	,000	,964
	Suma de cuadrados y productos vectoriales	367,506	-182,691	-1350,735	-5148902,750	42,686	-578,531
	Covarianza	3,868	-2,228	-15,891	-54198,976	,821	-20,662
	N	96	83	86	96	53	29
PCR	Correlación de Pearson	-,233*	1	,443**	,359**	-,437**	,036
	Sig. (bilateral)	,034		,000	,001	,002	,864
	Suma de cuadrados y productos vectoriales	-182,691	1873,073	3295,054	20391814,48	-76,678	4838,835
	Covarianza	-2,228	22,842	43,356	248680,664	-1,667	201,618
	N	83	83	77	83	47	25
VSG	Correlación de Pearson	-,387**	,443**	1	,525**	-,287*	,052
	Sig. (bilateral)	,000	,000		,000	,043	,800
	Suma de cuadrados y productos vectoriales	-1350,735	3295,054	36202,558	134874827,5	-205,092	37188,538
	Covarianza	-15,891	43,356	425,912	1586762,677	-4,186	1487,542
	N	86	77	86	86	50	26
Plaquetas	Correlación de Pearson	-,196	,359**	,525**	1	-,082	-,129
	Sig. (bilateral)	,055	,001	,000		,560	,503
	Suma de cuadrados y productos vectoriales	-5148902,750	20391814,48	134874827,5	1,869E+12	-424441,509	-640399448
	Covarianza	-54198,976	248680,664	1586762,677	1,967E+10	-8162,337	-22871408,9
	N	96	83	86	96	53	29
Albumina	Correlación de Pearson	,603**	-,437**	-,287*	-,082	1	-,187
	Sig. (bilateral)	,000	,002	,043	,560		,522
	Suma de cuadrados y productos vectoriales	42,686	-76,678	-205,092	-424441,509	23,201	-737,814
	Covarianza	,821	-1,667	-4,186	-8162,337	,446	-56,755
	N	53	47	50	53	53	14
Calprotectina fecal	Correlación de Pearson	-,009	,036	,052	-,129	-,187	1
	Sig. (bilateral)	,964	,864	,800	,503	,522	
	Suma de cuadrados y productos vectoriales	-578,531	4838,835	37188,538	-640399448	-737,814	80144146,47
	Covarianza	-20,662	201,618	1487,542	-22871408,9	-56,755	2428610,499
	N	29	25	26	29	14	34

*. La correlación es significativa en el nivel 0,05 (2 colas).

**. La correlación es significativa en el nivel 0,01 (2 colas).

Bivariate correlations in controls:

Significant positive correlations were observed between ($p<0.05$): plaquetas and CRP (r= 0.2), calprotectin and albumin (r= 0.6). Significant positive correlations were observed between ($p<0.01$):albumin and haemoglobin (r=0.6), ESR and CRP (r= 0.3), platelets and ESR (r= 0.4). Significant negative correlations were not observed with $p<0.05$. Significant negative correlations were observed between ($p<0.01$): haemoglobin and CRP (r= -0.3), ESR and haemoglobin (r= -0.3), platelets and haemoglobin (r= -0.4), albumin and ESR (r= -0.4)

Correlaciones

		Hemoglobina	PCR	VSG	Plaquetas	Albumina	Calprotectina fecal
Hemoglobina	Correlación de Pearson	1	-,308**	-,391**	-,407**	,687**	,038
	Sig. (bilateral)		,007	,001	,000	,000	,861
	Suma de cuadrados y productos vectoriales	307,699	-231,016	-1805,096	-8306181,815	42,573	860,475
	Covarianza	3,996	-3,122	-25,071	-107872,491	1,183	37,412
	N	78	75	73	78	37	24
PCR	Correlación de Pearson	-,308**	1	,399**	,241*	-,300	-,041
	Sig. (bilateral)	,007		,001	,037	,075	,852
	Suma de cuadrados y productos vectoriales	-231,016	1924,379	4513,700	10549919,34	-53,623	-2424,215
	Covarianza	-3,122	26,005	63,573	142566,478	-1,532	-110,192
	N	75	75	72	75	36	23
VSG	Correlación de Pearson	-,391**	,399**	1	,478**	-,455**	-,113
	Sig. (bilateral)	,001	,001		,000	,005	,599
	Suma de cuadrados y productos vectoriales	-1805,096	4513,700	71027,561	130836542,9	-496,095	-46650,208
	Covarianza	-25,071	63,573	986,494	1817174,207	-14,174	-2028,270
	N	73	72	73	73	36	24
Plaquetas	Correlación de Pearson	-,407**	,241*	,478**	1	-,286	-,357
	Sig. (bilateral)	,000	,037	,000		,086	,087
	Suma de cuadrados y productos vectoriales	-8306181,815	10549919,34	130836542,9	1,355E+12	-1107792,189	-397166599
	Covarianza	-107872,491	142566,478	1817174,207	1,759E+10	-30772,005	-17268113,0
	N	78	75	73	78	37	24
Albumina	Correlación de Pearson	,687**	-,300	-,455**	-,286	1	,657*
	Sig. (bilateral)	,000	,075	,005	,086		,039
	Suma de cuadrados y productos vectoriales	42,573	-53,623	-496,095	-1107792,189	20,890	1107,580
	Covarianza	1,183	-1,532	-14,174	-30772,005	,580	123,064
	N	37	36	36	37	37	10
Calprotectina fecal	Correlación de Pearson	,038	-,041	-,113	-,357	,657*	1
	Sig. (bilateral)	,861	,852	,599	,087	,039	
	Suma de cuadrados y productos vectoriales	860,475	-2424,215	-46650,208	-397166599	1107,580	3931448,962
	Covarianza	37,412	-110,192	-2028,270	-17268113,0	123,064	157257,958
	N	24	23	24	24	10	26

**. La correlación es significativa en el nivel 0,01 (2 colas).

*. La correlación es significativa en el nivel 0,05 (2 colas).

Faecal calprotectin

We have had an important limitation with the small sample size, moreover, we did not have samples in some episodes. Nevertheless, there is a tendency a higher levels in CDI patients group (cases). Calprotectin levels show a tendency to be higher (>500) in cases.

	CDI episodes	**Controls**	**p**
	N=34	N=26	
Mean	992.47	464.96	NS
Standard deviation	1558.400	396.558	

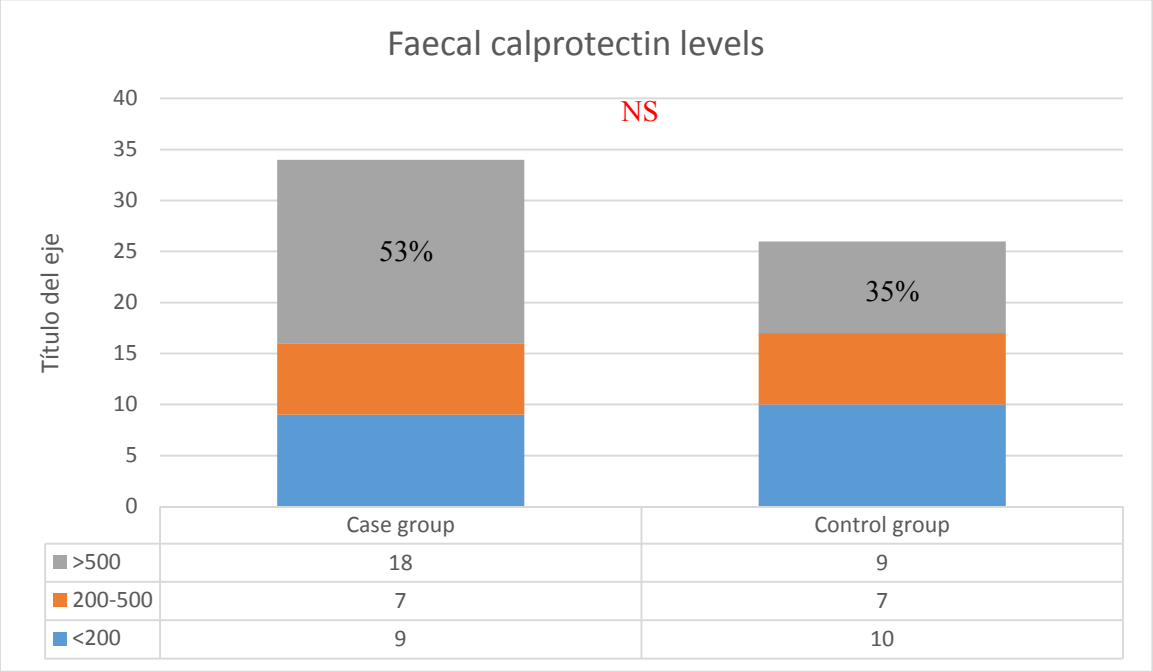


Figure. Faecal calprotectin level in cases and controls

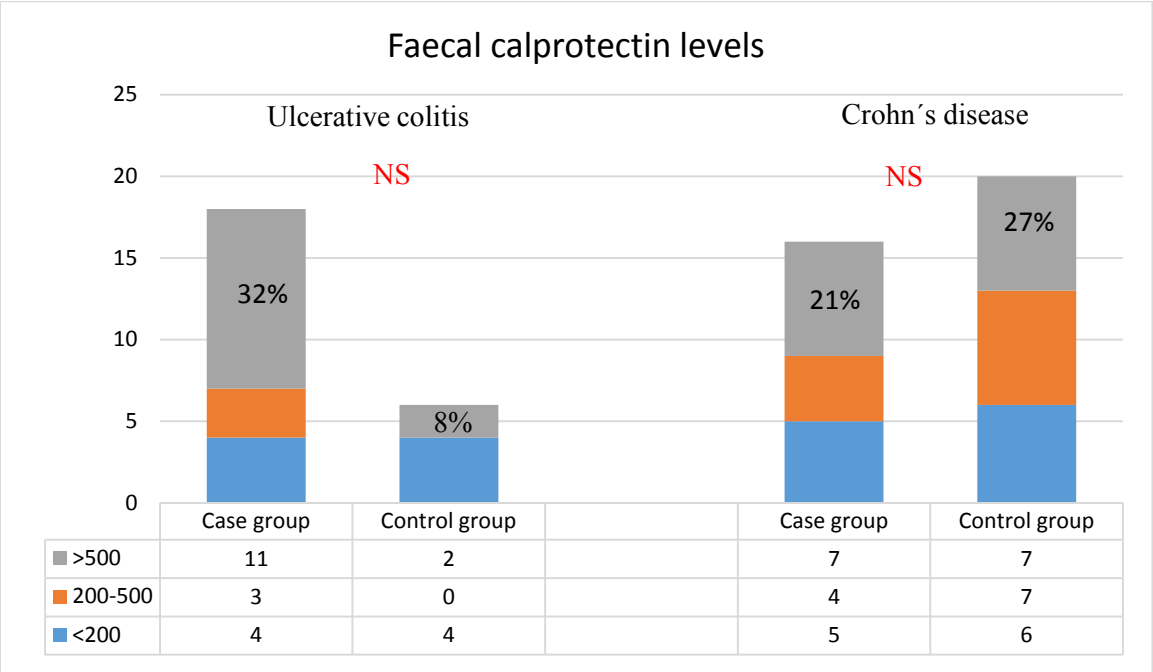


Figure. Faecal calprotectin levels in UC and CD patients in cases and controls

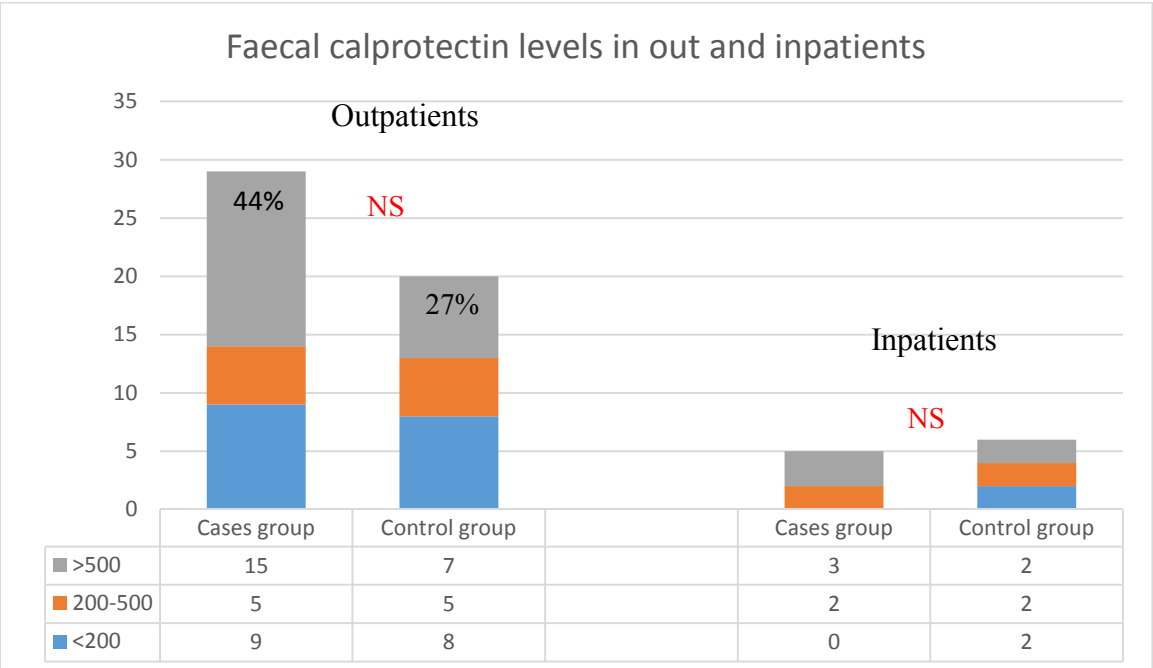


Figure. Faecal calprotectin levels in out and inpatients.

We found a tendency to higher calprotectin levels and clinical severity in our samples: in mild episodes the values had a tendency < 200, in moderate episodes, had a tendency >500 and in severe episode we did not have samples to evaluate. The right determination of this parameter in our study had two limitations: small sample size and severity definition.

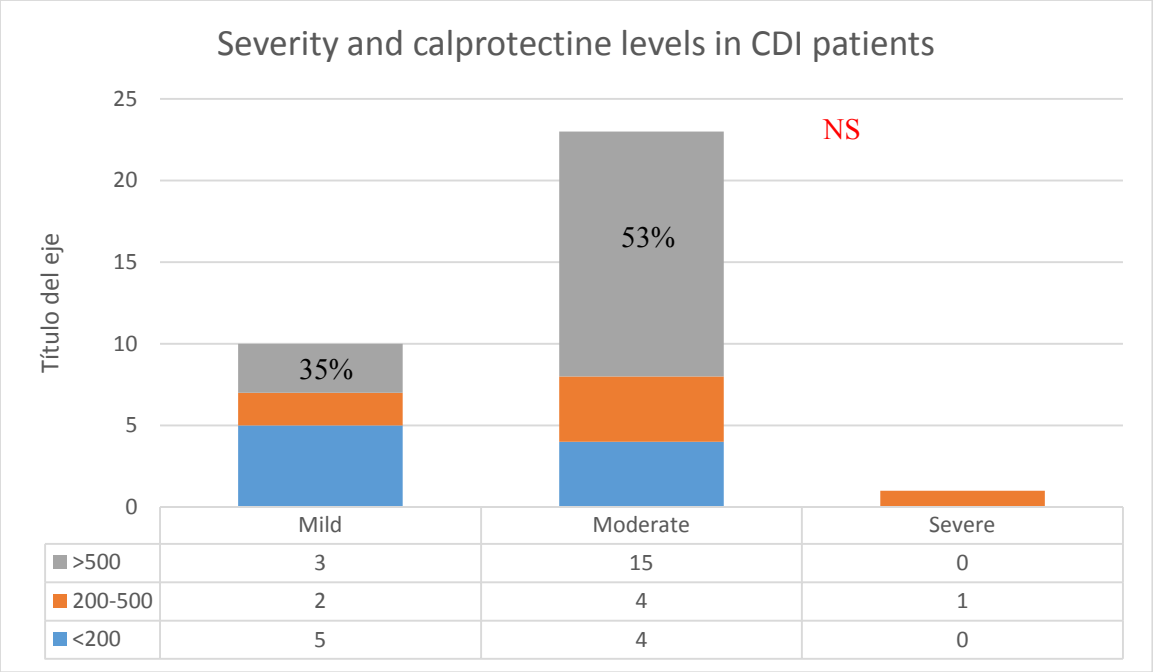
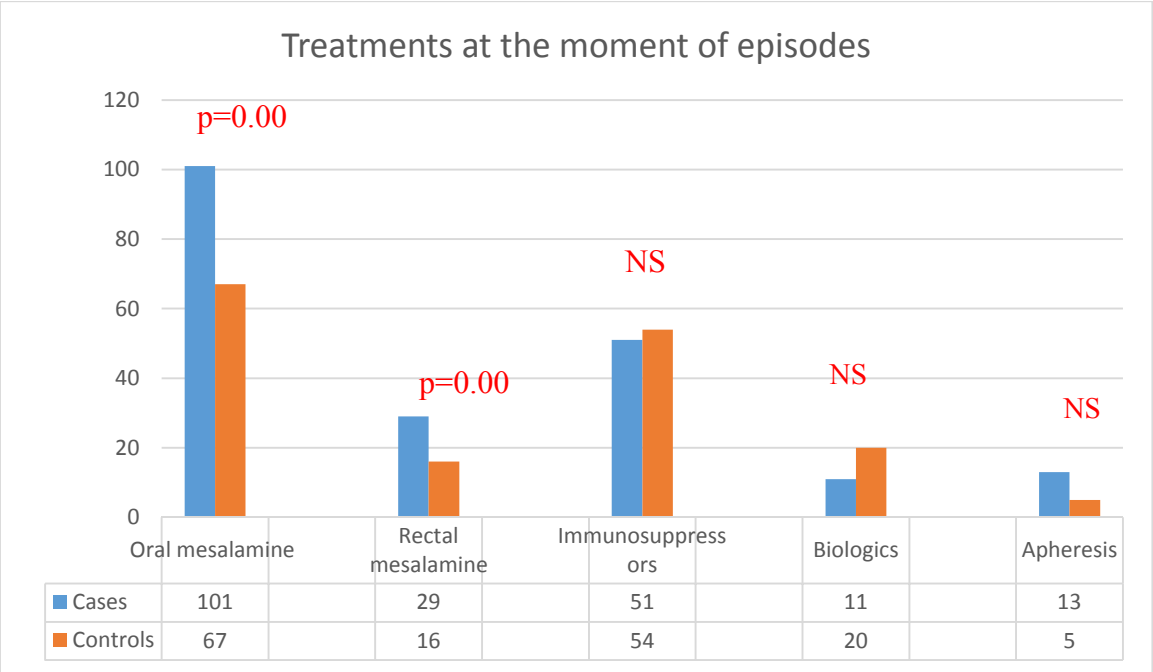
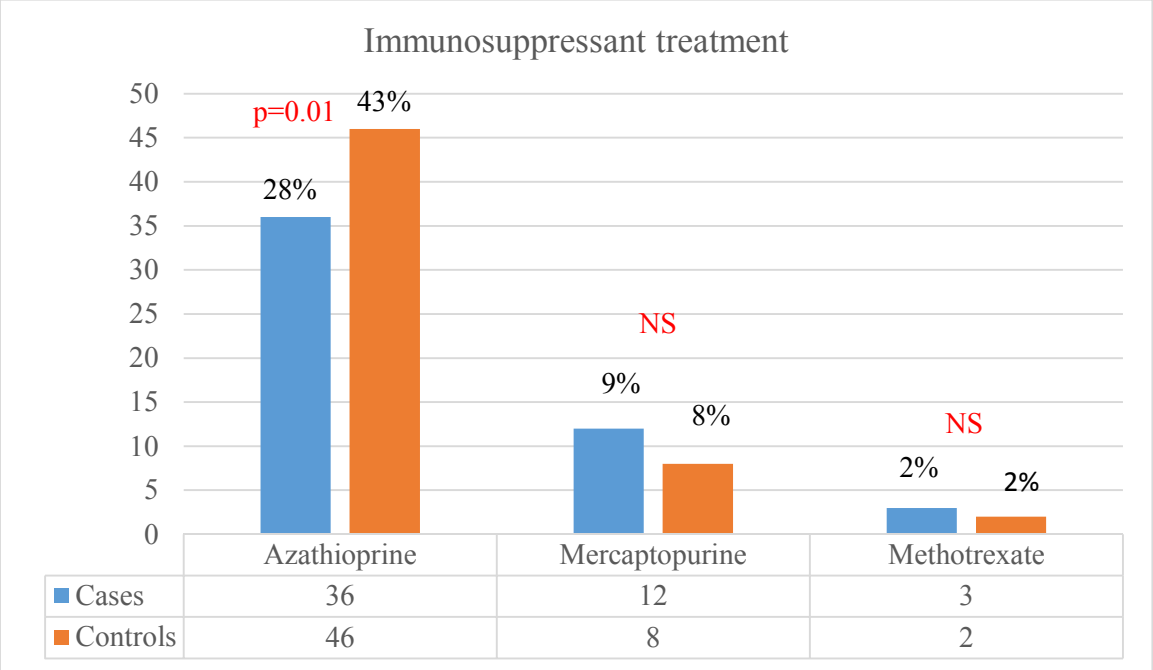


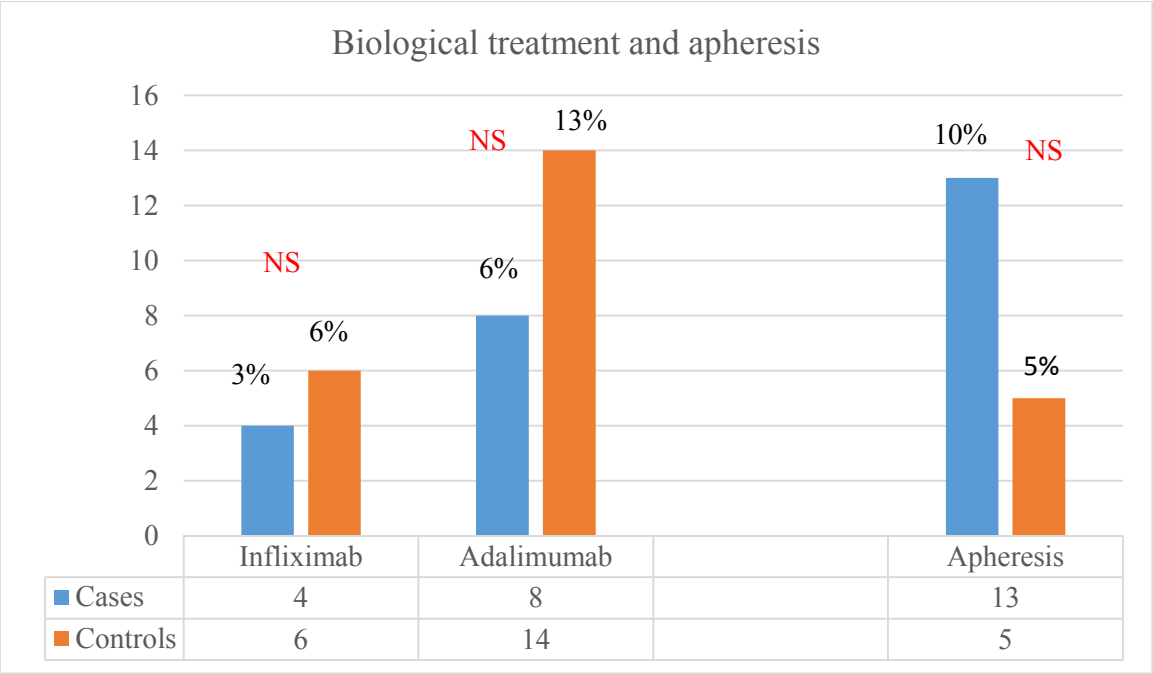
Figure. Severity and calprotectin levels

4.3.3 Treatments at the moment of episodes

The results were in cases and controls respectively: Oral mesalamine; 77% (101) and 63% (67) (p=0.00); 73% (74) of episodes in cases were in UC (p=0.00) and 52% (35) of episodes in controls are in UC too. Rectal mesalamine: 34% (29) and 39 (16); 93% (29) of episodes in cases were in UC (p=0.00) and 88% (16) of episodes in controls are in UC too (p=0.00). We did not find differences in the use of immunosuppressant treatment in cases and controls: 39% (51) and 50% (54) but controls were treated more with azathioprine (OR 1.43 95% CI 1.09-1.88 p=0.01). Biological treatment: 8% (11) and 19% (20) and apheresis treatment: 10% (13) and 5% (5) without differences between two groups.


Treatments at the moment of episodes
120
100
80
60
40
20
0
p=0.00
p=0.00
NS
NS
NS
Oral mesalamine
Rectal mesalamine
Immunosuppressors
Biologics
Apheresis
Cases 101 29 51 11 13
Controls 67 16 54 20 5


Immunosuppressant treatment
50
45
40
35
30
25
20
15
10
5
0
p=0.01
28%
43%
NS
9%
8%
NS
2%
2%
Azathioprine
Mercaptopurine
Methotrexate
Cases 36 12 3
Controls 46 8 2



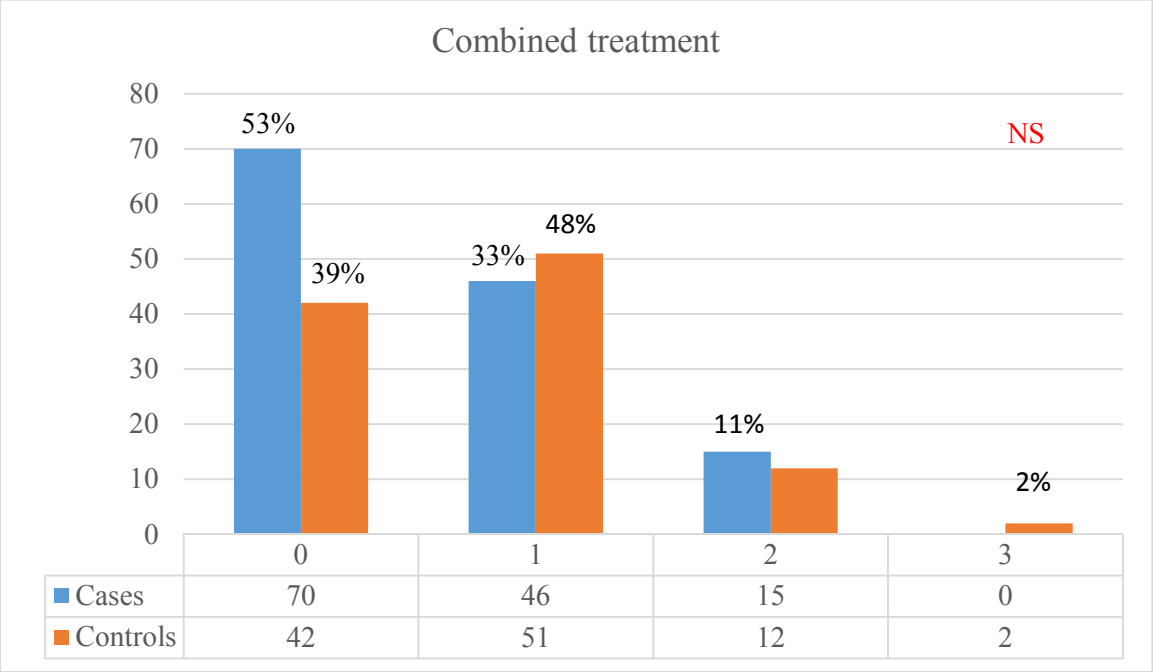


Figure. Combined treatment: 0, 1 (immunosuppressant or biological treatment or apheresis), 2 (immunosuppressant y/o biological treatment y/o apheresis), 3 (immunosuppressant, biological and apheresis treatment)

4.3.4 Risk factors

Antibiotics 3 months before comparing cases and controls: 16% (21) and 6% (6) (OR 1.49; CI 95% 1.17-1.89, p=0.01). In control group: UC y CD 0% y 100% respectively (p=0.04). PPIs 3 months before: 63% (82) and 45% (48) (OR 1.39; CI 1.08-1.77, p=0.00). Hospitalization 3 months before: 7% (9) and 9% (10). Surgery 3 months before: 3% (4) and 7% (8).

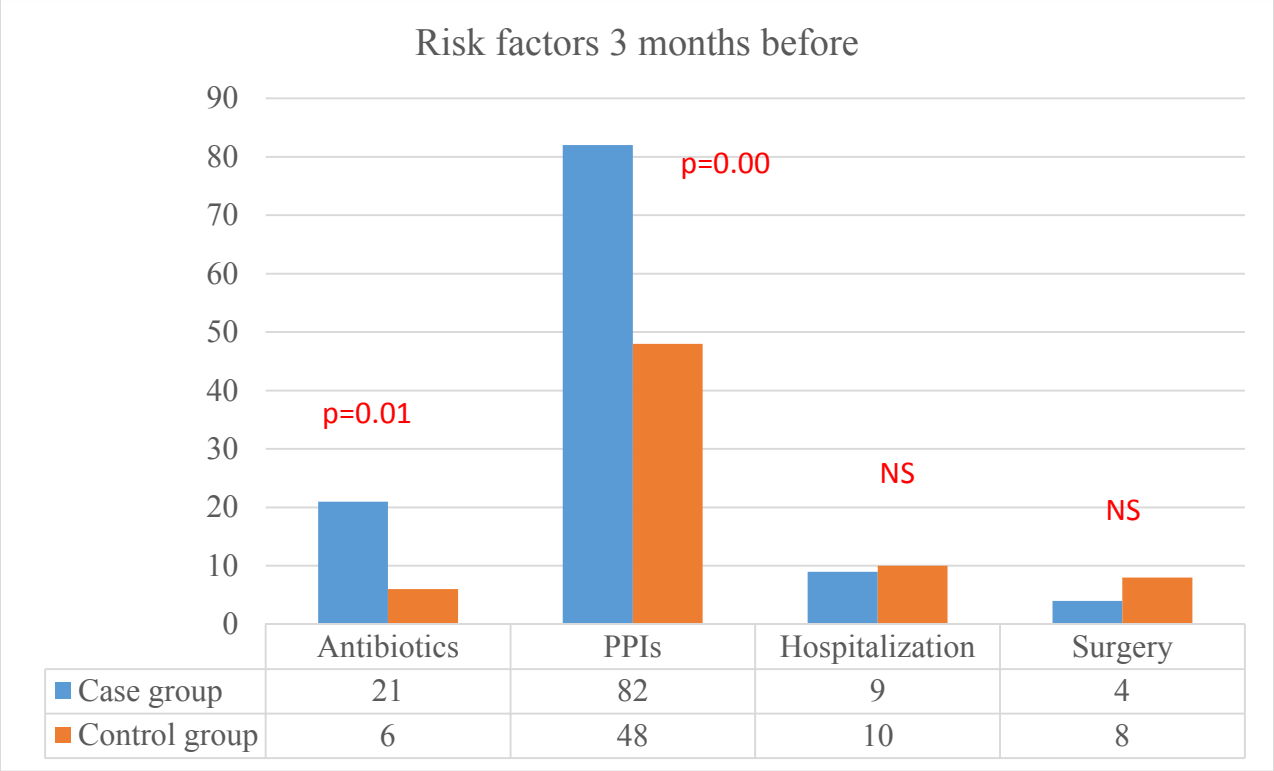


Figure. Distribution of main risk factors at the moment of IBD relapse with/without CDI (case and control group).

We found in the group of hospitalized patients PPIs (OR 2.63; CI 95% 1.28-5.39, p=0.00) and antibiotics 3 months before episodes (OR 1.66; CI 95% 1.06-2.58, p=0.05) were significantly more frequent in CDI patients comparing cases and controls.

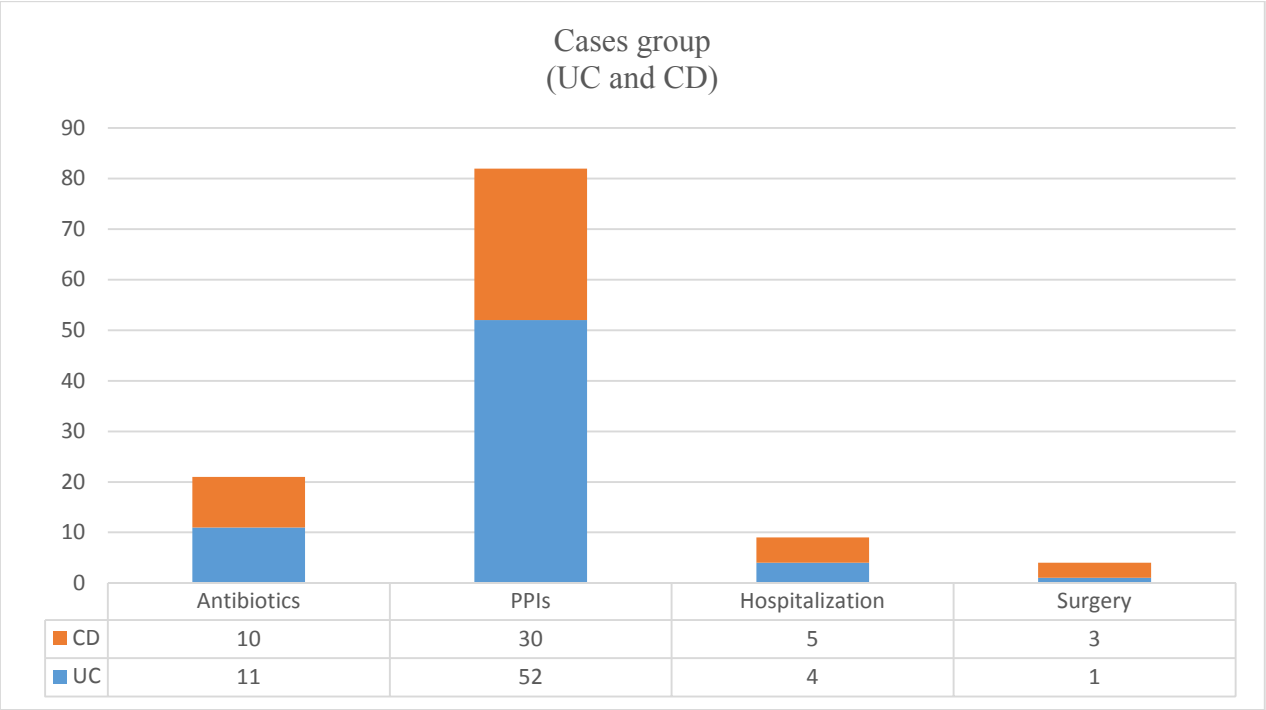


Figure. Distribution of main risk factors in UC and CD in case group.

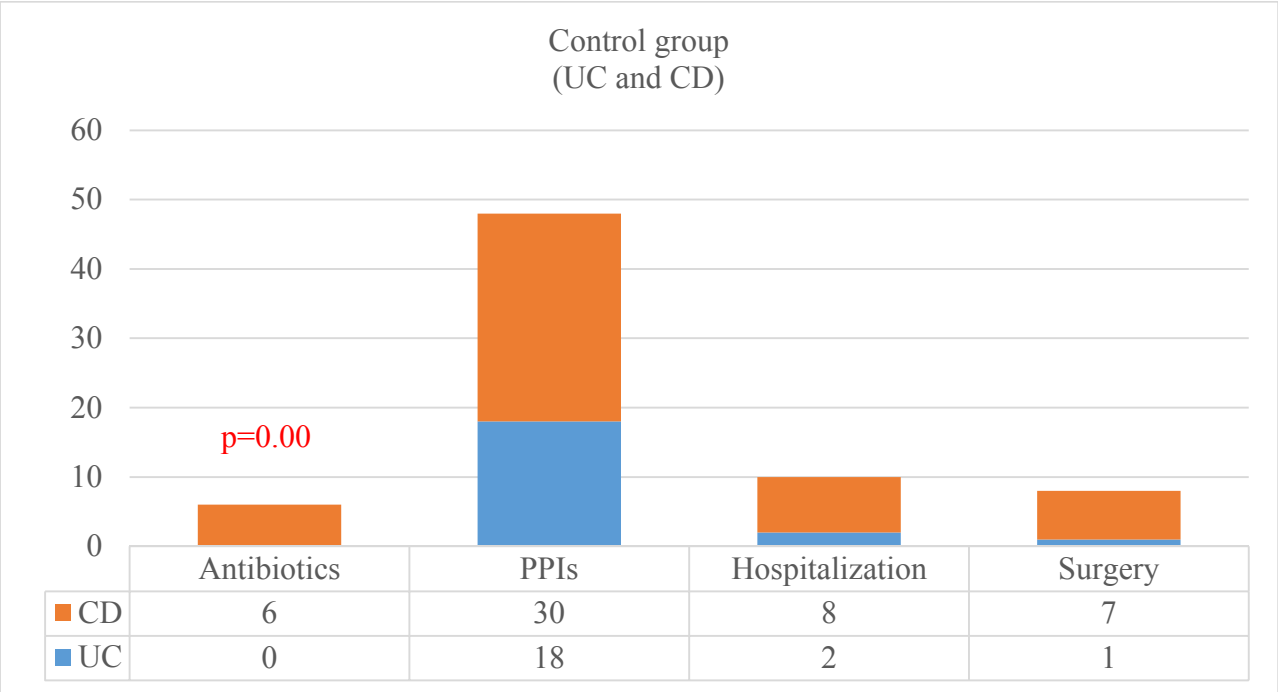


Figure. Distribution of main risk factors in UC and CD in controls group.

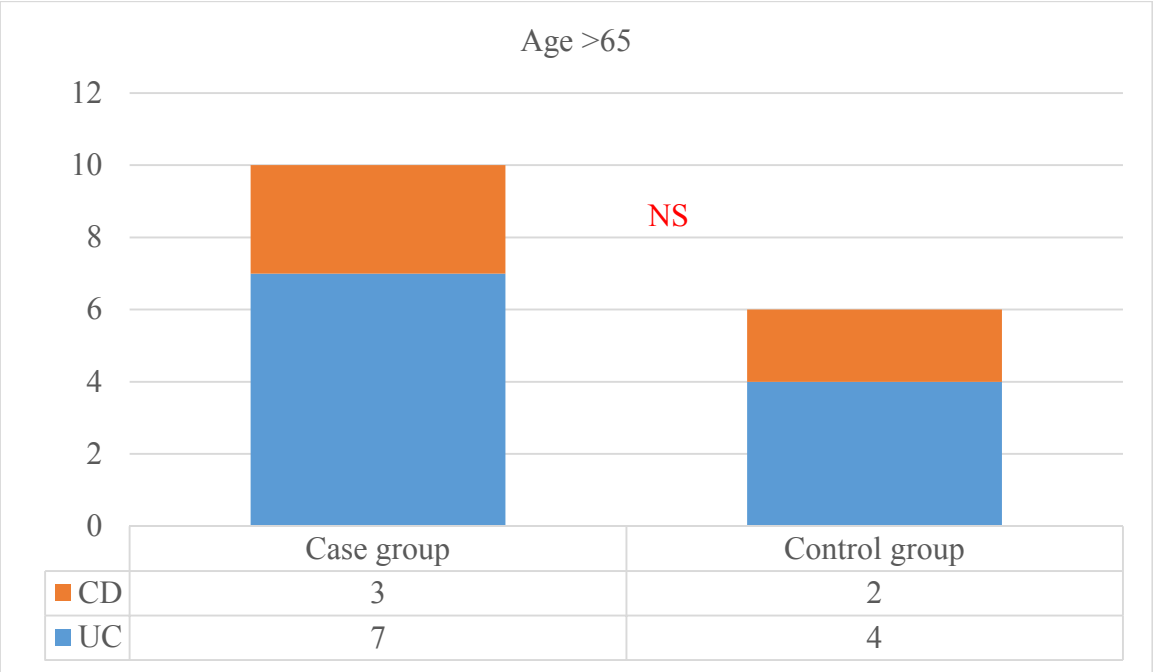


Figure. Age >65

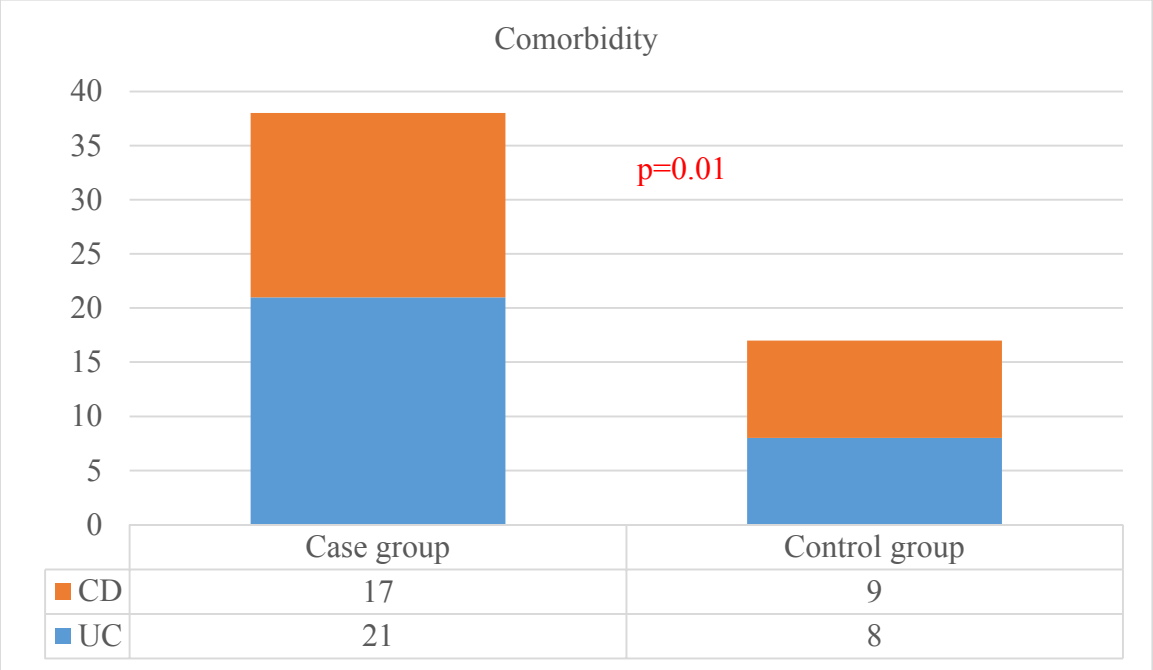
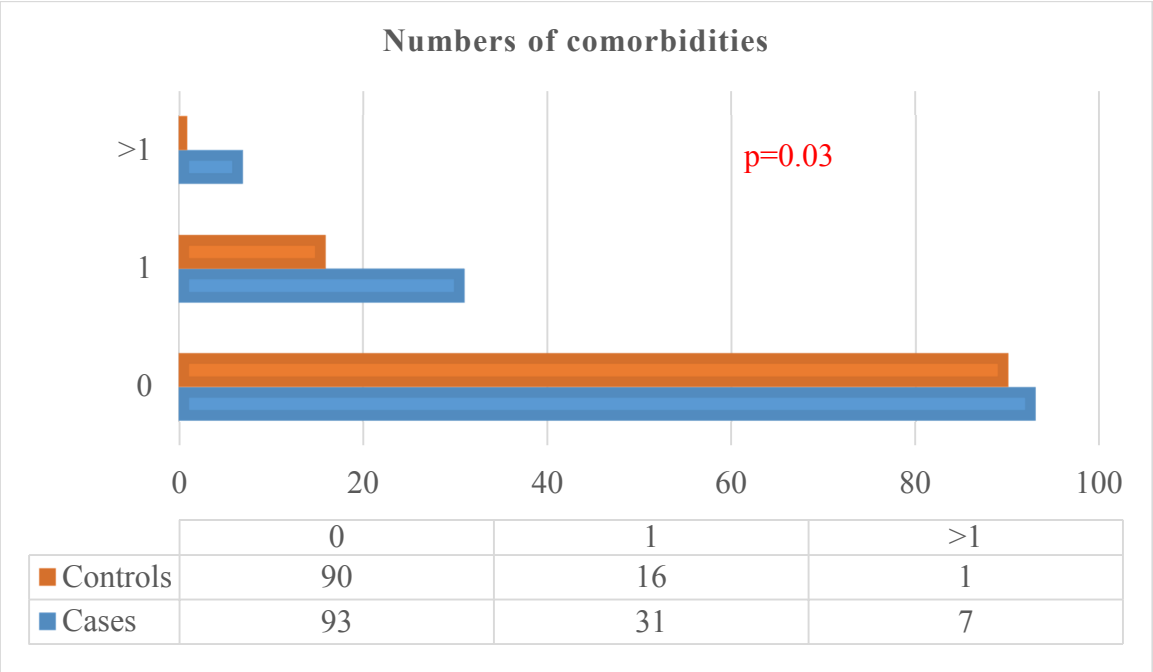


Figure. Comorbidity comparing cases and controls



	0	1	>1
Controls	90	16	1
Cases	93	31	7

Figure. Numbers of comorbidities

4.3.5 Independent risk factors for CDI episodes

We performed the multivariate analysis with variables with statistical signification or p<0.2 in univariate analysis.

Variables en la ecuación

		B	E.T.	Wald	gl	Sig.	Exp(B)	I.C. 95% para EXP(B)	
								Inferior	Superior
Paso 4[a]	InfeyDx(1)	,981	,502	3,818	1	,051	2,666	,997	7,129
	Antibprevi(1)	1,430	,545	6,884	1	,009	4,178	1,436	12,158
	tipoenf(1)	1,155	,305	14,300	1	,000	3,173	1,744	5,772
	Comorbilidad(1)	,934	,357	6,857	1	,009	2,544	1,265	5,118
	Constante	-3,471	,796	19,023	1	,000	,031		

a. Variable(s) introducida(s) en el paso 4: InfeyDx.

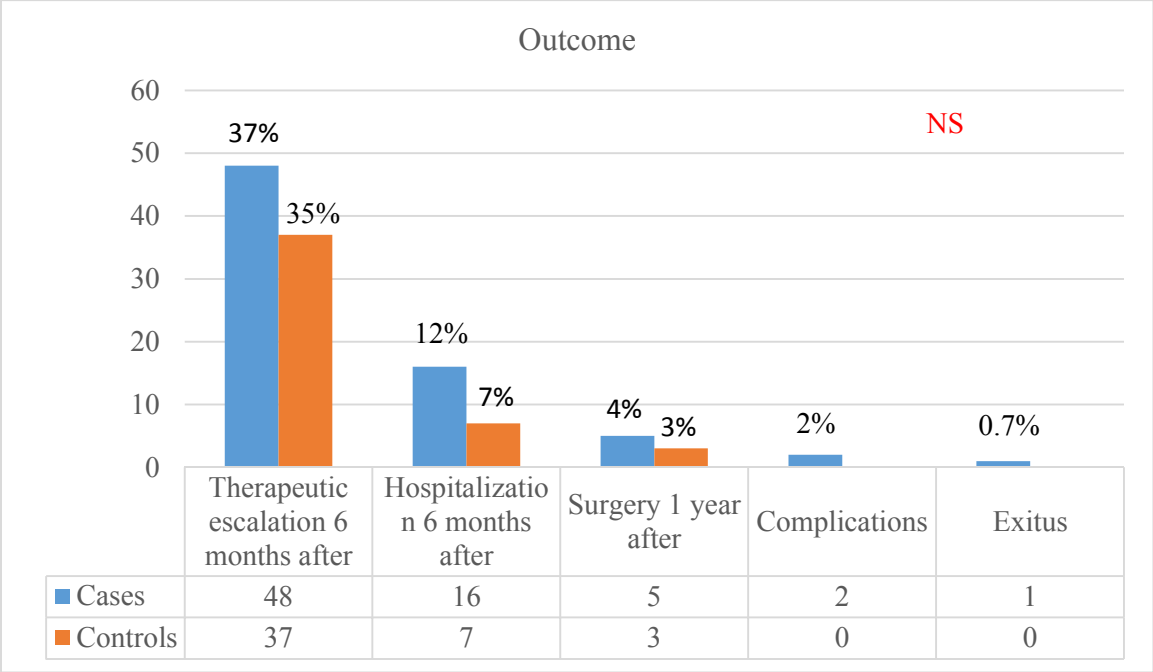
Risk profile of IBD patients with a relapse for CDI (independent risk factors in multivariate analysis):

- Patients with ulcerative colitis.
- Medical history of antibiotics 3 months before episodes.
- IBD diagnosis and CDI at the same time.
- Comorbidity.

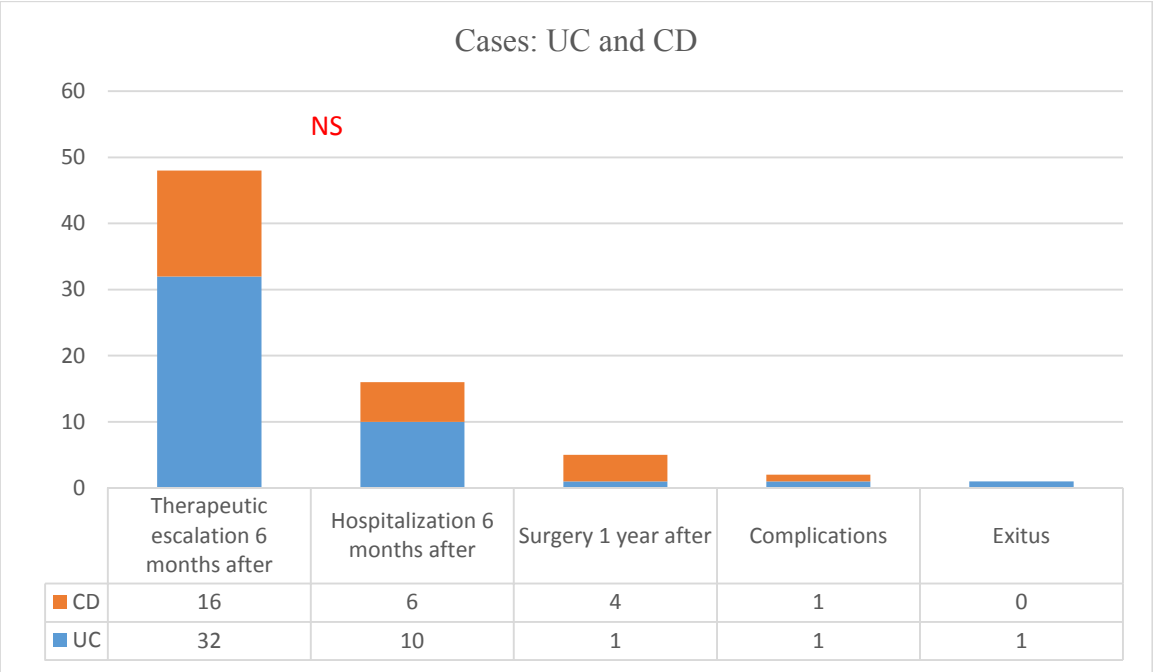
(PPIs 3 months before episodes, were a risk factor in univariate analysis)

4.3.6 Outcome

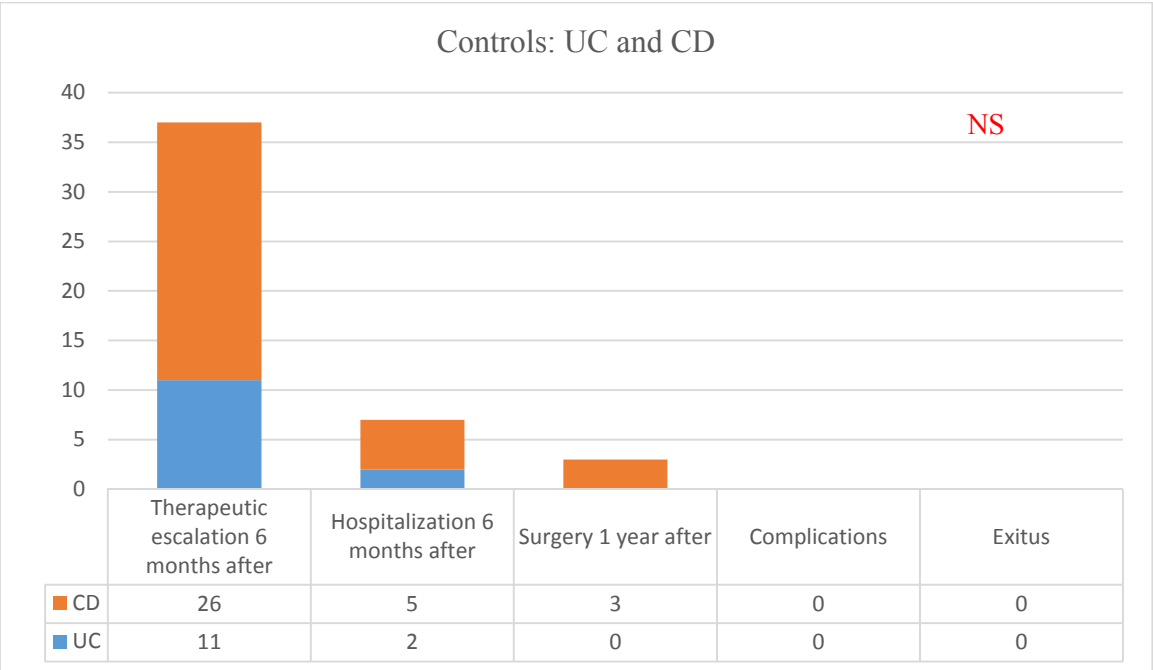
We did not find differences in the outcome between cases and controls: therapeutic escalation 6 months after episodes: 37% (48) and 35% (37); hospitalization 6 months after episodes: 12% (16) and 7% (7); surgery 1 year after episodes: 4% (5) and 3% (3); complications: 2% (2) and death 0.7% (1). We had 2 episodes with complications and 1 death.



	Therapeutic escalation 6 months after	Hospitalization 6 months after	Surgery 1 year after	Complications	Exitus
Cases	48	16	5	2	1
Controls	37	7	3	0	0



	Therapeutic escalation 6 months after	Hospitalization 6 months after	Surgery 1 year after	Complications	Exitus
CD	16	6	4	1	0
UC	32	10	1	1	1



	Therapeutic escalation 6 months after	Hospitalization 6 months after	Surgery 1 year after	Complications	Exitus
CD	26	5	3	0	0
UC	11	2	0	0	0

4.3.7 CDI treatments

We treated 97% of episodes (first and recurrent ones) with oral metronidazole as the first choice. In general, we did not use oral vancomycin, and we used spiraxin in some first episode. We did not use probiotics except in one recurrent episode, and we did not use spiraxin as coadjuvant treatment.

	Global (n=131)	**Ulcerative colitis (n=85)**	**Crohn´s disease (n=46)**	**p**
Metronidazole (oral)	127 (97%)	83 (98%)	44 (96%)	NS
First episode (n=107)	104 (97%)			
Recurrent episodes (n=24)	23 (96%)			
Vancomycin (oral)	2 (2%)	1 (1%)	1 (0.2%)	
First episode (n=107)	1 (0.9%)			
Recurrent episodes (n=24)	1 (4 %)			
Metronidazole (IV)	1 (0.7%)	1 (1 %)	0	
First episode (n=107)	1 (0.9%)			
Recurrent episodes (n=24)	0			
Spiraxin	4 (3%)	2/85 (2.3%)	2/46 (4.3%)	
First episode (n=107)	4 (4%)			
Recurrent episodes (n=24)	0			
VSL 3	1/131 (0.7%)	1/85 (1%)	0	

First episode (n=107)	0
Recurrent episodes (n=24)	1 (4%)

Corticosteroids were used in 71% (91) of episodes. Prednisone 76% (69) and beclomethasone 23% (21). Mean dose of prednisone: 43 mg (10-60) and beclomethasone: 9 mg (5-10). We did not find differences with control group.

Table. Corticoids treatment in CDI episodes

Corticosteroids	91 (71%)
Prednisone	69 (76%)
Mean doses: 42.91+/-10.806 (10-60 mg)	
Beclometasone	21 (23%)
Mean doses: 9.32+/-7.286	
Budesonide	1 (1%)

4.3.8 Analysis in the subgroup of patients with age > 65

We analyzed 10 episodes (8%) and 6 episodes (7%) in case and control group respectively. Less than 10% of episodes occurred in patients with age >65 in both groups. We could not find differences between cases and controls due to the small sample size. Even so, this subgroup of patients has a very interesting behavior, different to the general population.

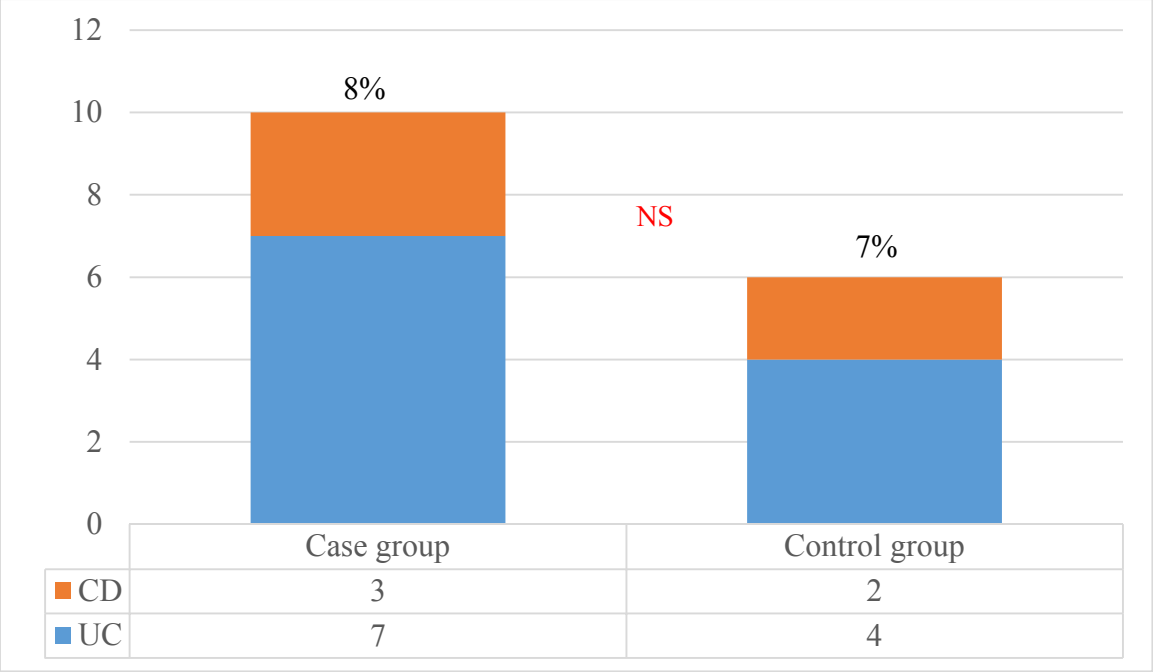


Figure. Patients with age>65

Mean age was more than 70 years old at the moment of episodes. No differences were found regarding gender, seasonality and smoking history. Most of them did not have comorbidities, only 1 out of 3 had more than one comorbidity in cases. The diagnosis was done as outpatients more than 70% in both groups. There were no differences in the percentage of hospitalization and average stay in both groups (See table below).

Table. Episodes characteristics

	Cases	**Controls**	**p value**
Episodes	n=10 (8%)	n=7 (7%)	NS
Mean age	73.20 +/-6.426	74.17 +/-6.775	NS
Gender			
Female	2 (20%)	4 (57%)	p=0.05
Male	8 (80%)	2 (29%)	
Autumn-Winter	1 (10%)	1 (14%)	NS
Smoking history	2 (20%)	0	NS
Comorbidities	4 (40%)	5 (71%)	NS
0	6 (60%)	2 (29%)	
1	1 (10%)	4 (57%)	
>1	3 (30%)	1 (14%)	
Outpatient	8 (80%)	5 (71%)	NS
Hospitalization at the moment of episode	2 (20%)	2 (29 %)	NS
Mean stay (days)	6.00±1.414	9.00±6.245	NS

In our study, UC was more frequent in cases and controls. Mean age at IBD diagnosis was an older age too and the time of IBD evolution until episodes were only 3-4 years (similar to episodes in <65). Thus, episodes occurred more frequently during the first 3-4 years after IBD diagnosis.

IBD diagnosis and relapses occurred at the same time in 20% of episodes and half of them needed hospitalization at that moment. In general, 20% of episodes needed hospitalization during episodes and half were relapses at the time of IBD diagnosis.

First episodes were 70% (7) but recurrent episodes were 30% (3) **(**1 out of 3) and more than 1 recurrence in 67% (2) of episodes. We found 10 % of first, second and third recurrent episodes.

Table. IBD characteristics

	Cases	**Controls**	**p value**
Disease			
UC	7 (70%)	4 (57%)	NS
EC	3 (30%)	2 (29%)	
Mean age at IBD diagnosis	69.00+/-4.989	68.71+/-6.775	NS
Age at diagnosis of CDI			
≤16	**0**	**0**	
17-40	**0**	**0**	
>40	**10 (100%)**	**7 (100%)**	NS
Time of IBD evolution (years)	3.70 +/-3.917	4.00 +/-4.775	NS
IBD diagnosis and relapse at the same time	2 (20%)	1 (14%)	NS
Relapse at the moment of IBD diagnosis and hospitalization	1 (10%)	0	NS
First episode CDI	7 (70%)		
Recurrence episodes	3 (30%) (>1 recurrencia 2 episodes 67%)		
First recurrence (8 months)	1 (10%)		
Second recurrence	1 (10%)		
Third recurrence	1 (10%)		

Proctitis (E1) was most frequent in UC episodes with CDI. Extensive localization was most common in controls. Left localization was similar in both groups. Ileal localization was two times more frequent in the control group, and the colonic involvement was two times more common in cases. Stricturing behaviour was more predominant in controls (double) and non-stricturing non-penetrating behaviour in both. There were not any episodes in penetrating behaviour, abscesses or plastron. Therefore, in more than 65 years old patients there was a tendency to have less penetrating phenotype and its complications. In patients with CD, colonic involvement was more frequent in CDI episodes and ileum in the control group. The localization in small bowel only was presented in the control group.

Most of the episodes were moderate, and we had not severe ones. Comorbidity was similar in both groups, but the cases had a tendency for a higher number of comorbidities.

Table. IBD localization and severity

	Cases	**Controls**	**p value**
Crohn´s disease			
Localization			NS
L1	1 (10%)	2 (29%)	
L2	2 (20%)	0	
L3	0	0	
L4	0	1 (14%)	
Perianal	0	0	
Behavior			
B1	3 (30%)	3 (43%)	NS
B2	1 (10%)	2 (29%)	
B3	0	0	
Abscess	0	0	
Plastron	0	0	
Ulcerative colitis			
S1	4 (40%)	0	NS
S2	3 (30%)	2 (29%)	NS
S3	0	2 (29%)	NS
Severity			
Mild	4 (40%)		
Moderate	6 (60%)		
Severe	0		
Surgery	0	1	NS

Regarding treatment at the moment of episodes: in cases 70% were treated with oral mesalamine but only 40% were treated with rectal mesalamine. Cases were treated with immunosuppressant and biological treatment in 40% (4) and 30% (3) respectively, compared with controls 14% (1) and 29% (2). Immunosuppressant treatment was used in CDI patients four times more than in control group. Biological treatment was utilised in the same proportion in both groups, 30% (3).

A combination of immunosuppressant +/- biological treatment were not used in more than 50% of patients in total; but 2 combined treatment was more frequent in cases, and 1 immunosuppressant or biological treatment was more common in controls.

Table. IBD treatment

	Cases	**Controls**	**p value**
Oral mesalamine oral	7 (70%)	4 (57%)	NS
Rectal mesalamine	4 (40%)(60% no)	1 (14%)	NS
Immunosuppressors	4 (40%)	1 (14%)	NS
Azathioprine	0	1 (14%)	
Biological treatment	3 (30%)	2 (29%)	NS
Apheresis	0	0	
Combined treatment (Immunosuppressors and/or biologics and/or apheresis)			NS
0	6 (60%)	4 (57%)	
1	1 (10%)	3 (50%)	
2	3 (30%)	0	
3	0	0	

This subgroup of patients in our short series did not have the same risk factor compared with general population. They did not take antibiotics, were not hospitalized or surgery 3 months before of episodes. However, PPIs treatment was more frequent in CDI episodes compared with controls (90% vs. 42%).

Table. Risk factors

	Cases	**Controls**	**p value**
Antibiotics 3 months before	0	0	NS

PPIs 3 months before	9 (90%)	3 (43%)	P=0.03
Hospitalization 3 months before	0	0	
Surgery 3 months before	1 (10%)	0	

Cases had a tendency for therapeutic escalation 6 months after infection in contrast to the control group. 40% of episodes in CDI group needed hospitalization 6 months after episodes while in the control group did not. Moreover, we found a 10% of surgeries in cases compared with 0% in controls.

It has seemed a tendency to the escalation of treatment, hospitalization, surgery, complications and mortality in CDI group in contrast with no episodes in the control group. All complications and mortality occurred in CDI episodes. It would be necessary to increase the number of patients for improving the statistic power of this study.

Table. Outcome

	Cases	**Controls**	**p value**
Escalation therapeutic 6 months after	3 (30%) (1/3)	0%	NS
Hospitalization 6 months after	4 (40%)	0%	NS
Surgery 1 year after	1(10%)	0%	NS
Complications	1 (10%)	0%	NS
CDI complications	0%	0%	
Exitus	1 (10%)	0%	

Laboratory parameters

	Cases	Controls	p value
Calprotectin levels	N=3	No	
<200	1 (10%)		
200-500	2 (20%)		
>500	0		
ESR	40.40	30.67	NS
CRP	1.5633	1.4833	
Albumin	3.36	3.400	
Platelets	327,333.33	346,500	
Hemoglobin	11.53	12.55	
Ferritin	96.80	105.73	
Fe	49.20	51.50	

We had a small sample size of episodes with laboratory parameter, but we did not find differences between both groups.

4.4 Recurrent episodes of CDI in IBD patients

So far, we have analysed from June 2007 to June 2015, 131 CDI episodes in IBD patients with a relapse (cases) compared with IBD patient with a relapse but without infection (controls). We found 24 recurrent episodes, and we are going to analyse their characteristics in this section.

First of all, we are going to show you a general perspective of them. Secondly, we are going to highlight their characteristics in general and specifically comparing UC and CD patients.

4.4.1 General picture of recurrent episodes in CDI patients

We found 24 (18%) recurrent CDI episodes. The distribution of recurrent CDI episodes from June 2007 to June 2015 showed a tendency to decrease in IBD patients (similar tendency to positive episodes in general). They were produced in UC patients more than in CD patients and more than 50% > 8 weeks after the previous episode. Last two years, there were not recurrent episodes in CD patients and the last year not recurrent episodes in both.

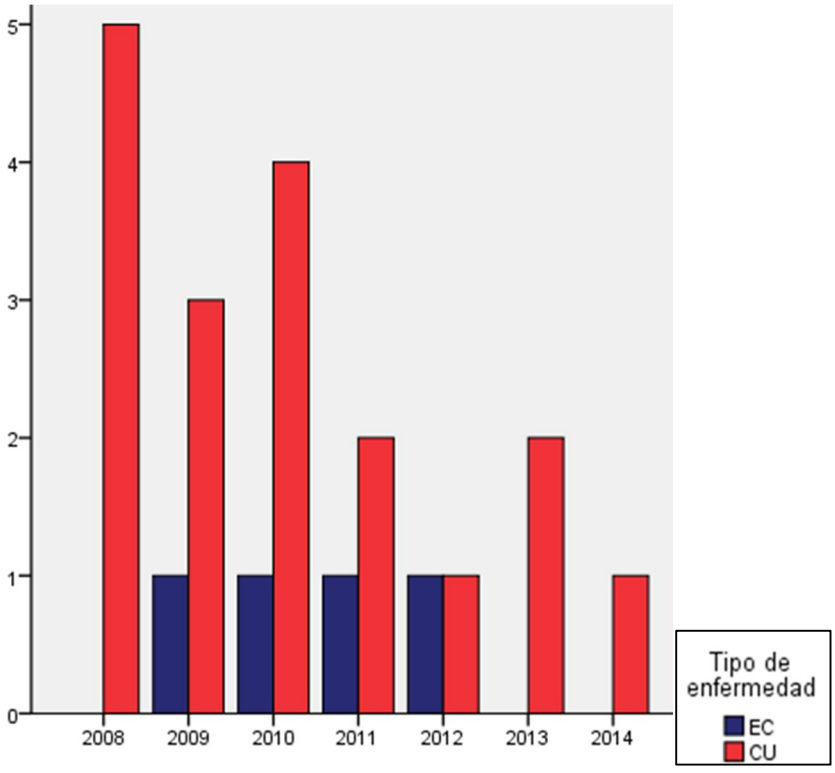


Figure. Distribution of recurrent CDI episodes from June 2007 to June 2015

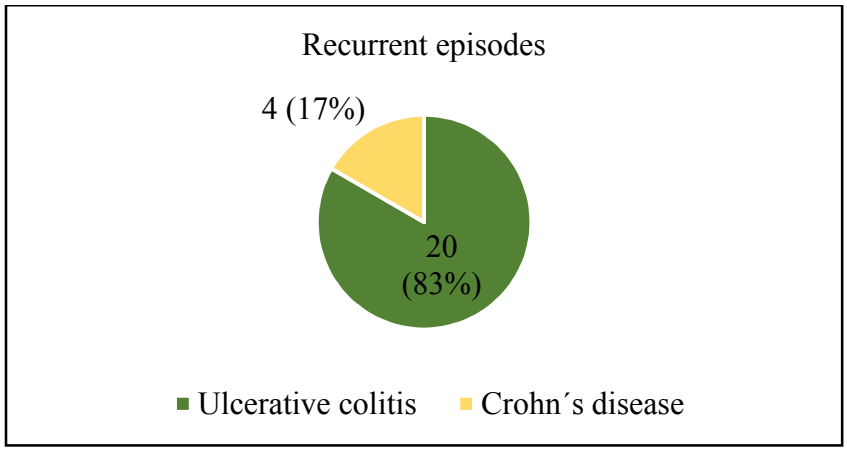


Figure. Distribution of IBD in recurrent episodes

In the analysis of recurrent episodes, the first recurrence was the most frequent, about 65% and more than 50% occurred >8 weeks after the previous episode. The first recurrence is more frequent than the second and the third one. However, the second recurrence was the earliest; it occurred <8 weeks after the previous one. In second and third recurrences, there were not CD episodes.

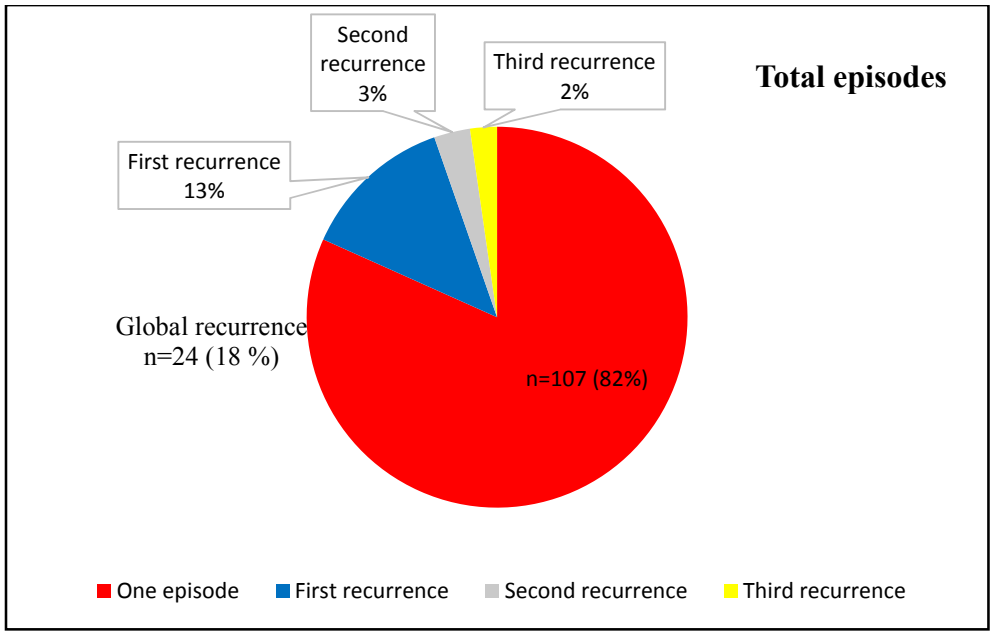


Figure. Distribution of recurrent episodes: first, second and third recurrences.

Table. The time it took for recurrent CDI episodes to appear in the patients.

Cases	n (%)	Recurrence time (months)	Recurrence time (> or <8 weeks)
Total CDI episodes	131		
Recurrent CDI episodes	24 (18%)		> 8 weeks 14 (58%) <8 weeks 10 (42%)
First recurrence	17 (71%)	17.57±21.964	>8 weeks 11 (65%)
Second recurrence	4 (17%)	9.00±13.663	>8 weeks 1 (25%)
Third recurrence	3 (13%)	21.33±24.132	>8 weeks 2 (67%)

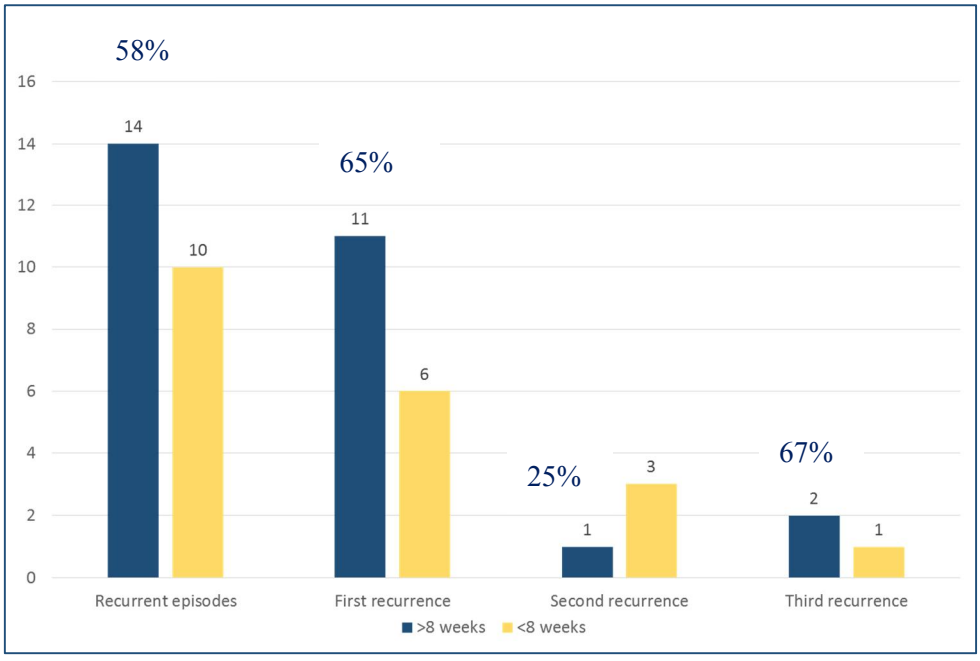


Figure. Time recurrence takes to appear (> or < 8 weeks)

Table. Recurrence time

	Recurrence time (months)
First recurrence	17.57±21.964
CD	27.00±21.055
UC	15.58±22.177
Second recurrence	9.00±13.663
CD	**No recurrences**
UC	9.00±13.663
Third recurrence	21.33±24.132
CD	**No recurrences**
UC	21.33±24.132

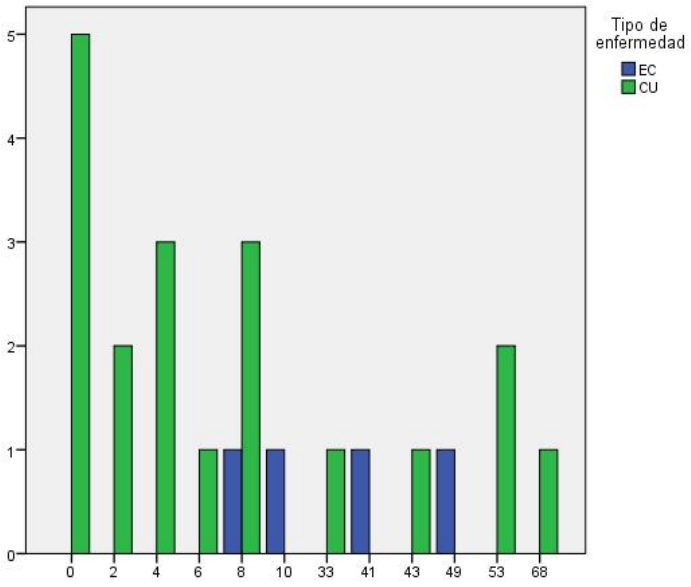


Figure. Distribution of UC and CD in the first recurrence

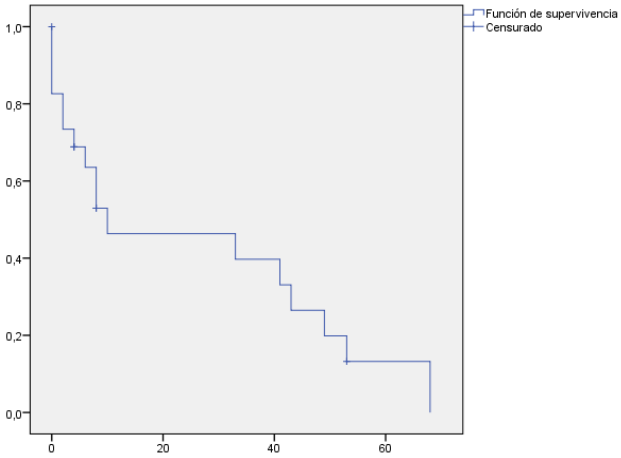


Figure. Probability of a first recurrence (months).

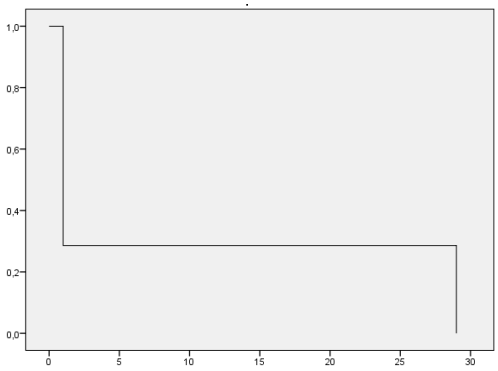


Figure. Probability of a second recurrence (months).

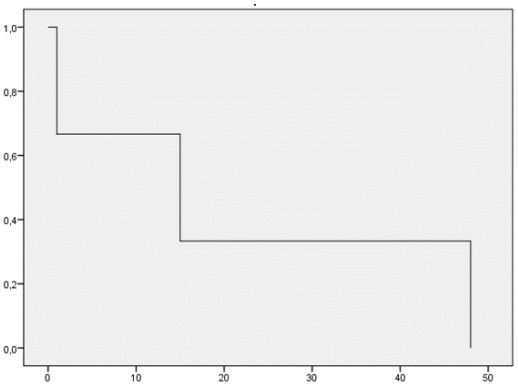


Figure. Probability of a third recurrence (months).

4.4.2 Characteristics of recurrent episodes and comparison of UC and CD

We analyzed 24 recurrent CDI episodes during the period of our study. 4 episodes (17%) occurred in CD patients and 20 episodes (83%) in UC patients (p=0.03).

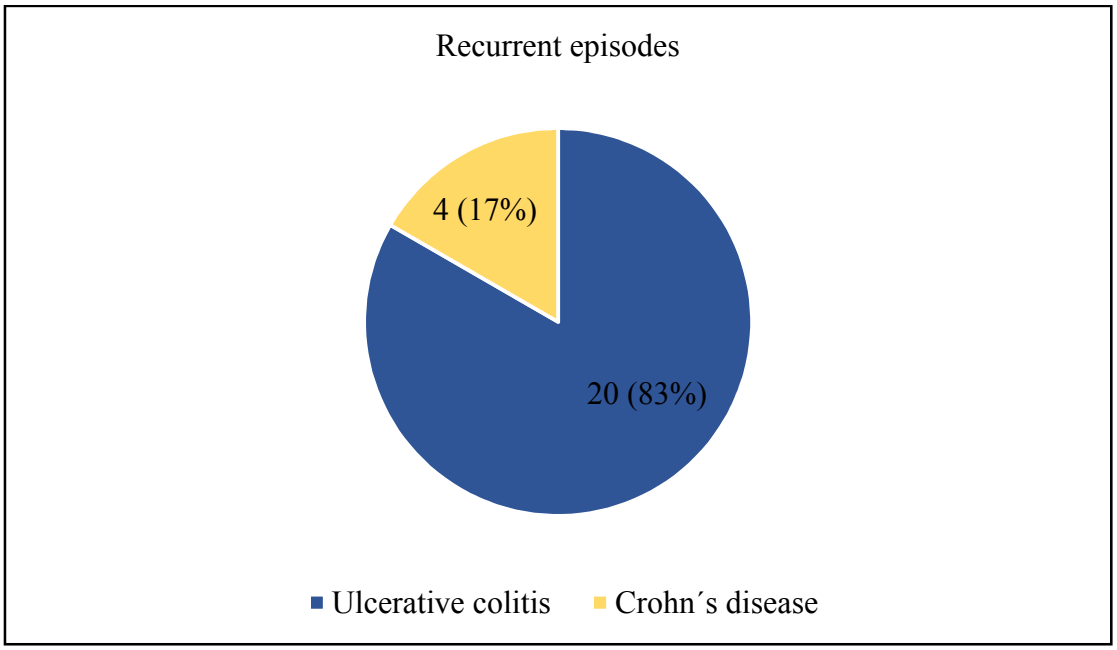


Figure. Distribution of IBD in recurrent episodes

We studied 24 recurrent episodes in which we found four episodes (17%) occurred in CD patients and 20 episodes (83%) in UC patients (p=0.03). IBD was diagnosed in middle-aged patients. The patients have a mean age of 39 at the moment of episodes, but 13% of recurrent episodes occurred in patients older than 65 and ulcerative colitis. In our study, we did not find patients >65 with a recurrent episode and Crohn´s disease. Women and men had a similar distribution. The recurrent CDI infections appeared in Autumn-Winter as well as in Spring-Summer. There is a smoking history in 13% of recurrent episodes and it is more frequent in the CD group (p=0.01). We found 21% of episodes with more than one comorbidity without differences between UC and CD. Recurrent episodes did not occur in patients with previous colectomy.

Table. General characteristics of patients with recurrent episodes

	Recurrent episodes (n=24)	**Ulcerative colitis (n=20)**	**Crohn´s disease (n=4)**	**p value**
Mean age±standard deviation	39.17+/-16.343	40.65+/-17.070	31.75+/-10.689	NS
Age >65	3 (13%)	3 (15%)	0%	NS
Gender				NS
Male	12 (50%)	9 (45%)	3 (75%)	
Female	12 (50%)	11 (55%)	1 (25%)	
Seasonality				NS
Autumn and Winter	10 (42%)	9 (45%)	1 (25%)	
Spring and Summer	14 (58%)	11 (55%)	3 (75%)	
Smoking history	3 (13%)	1 (5%)	2 (50%)	P=0.01
Comorbidity (≥1)	5 (21%)	3 (15%)	2 (50%)	NS
Surgery (colectomy)	0%	0%	0%	NS
Mean age at IBD diagnosis	33.21+/-17.118	35+/-17.859	24.25+/-9.912	NS

We found colonic involvement in 75% of episodes in Crohn´s disease. Left and extensive colitis localization are predominant in ulcerative colitis (35% and 40% respectively). Moreover, 50% of episodes in Crohn´s disease occurred in a disease with perianal localization too. We have found recurrent episodes with all behaviors without a prominent one.

Table. Disease localization and behavior in recurrent episodes

Disease localization in recurrent episodes (n=24)	Crohn´s disease (n=4)
L1	1 (25%)
L2	1 (25%)
L3	2 (50%)
L4	0 (0%)
Perianal	2 (50%)

Behaviour in recurrent episodes (n=24)	Crohn´s disease (n=4)
B1	1 (25%)
B2	1 (25%)
B3	1 (25%)
Plastron	1 (25%)
Abscess	1 (25%)

Table. Disease localization in recurrent episodes

Disease localization in recurrent episodes (n=24)	Ulcerative colitis (n=20)
E1 (proctitis)	5 (25%)
E2 (left-sided colitis)	7 (35%)
E3 (Extent colitis)	8 (40%)

88% of recurrent episodes happened in outpatients without differences between UC and CD. These recurrent episodes need hospitalization in 16% of episodes with more in the CD group (p=0.05) but with a mean stay of 12 days without differences between two groups. Most episodes (79%) are moderate episodes without differences between CD and UC.

Table. Characteristics of the recurrent episodes

	Recurrent episodes (n=24)	**Ulcerative colitis (n=20)**	**Crohn´s disease (n=4)**	
Outpatients	21 (88%)	18 (90%)	3 (75%)	NS
Hospitalization **Mean stay (days)** **Community acquired**	4 (17%) 12.00±8.756 (5-24) 100%	2 (10%) 9+/-5.657	2 (50%) 15+/-12.728	p=0.05
Severity **Mild** **Moderate** **Severe**	 4 (17%) 19 (79%) 1 (4%)	 4 (20%) 15 (75%) 1 (5%)	 0% 100% 0%	NS

More than 90% of recurrent episodes treated with oral mesalamine. This treatment with oral salicylates was administrated to 100% of UC patients and 50% of CD (p=0.00). It would be very important to say, 38% of recurrent episode treated with local mesalamine and 45% of UC episodes and 0% CD episodes with non-significant differences.

67% of recurrent episodes treated with immunosuppressive treatment and as well as 75% of the CD group and 65% of the UC group (p=0.01). Azathioprine and mercaptopurine were the most common immunosuppressive treatment used, in 58% of recurrent episodes and 65% of UC episodes (we use 50% azathioprine and 15% mercaptopurine in UC episodes if we consider them separately). We use more azathioprine treatment in UC compare to mercaptopurine. Methotrexate used in 8% of recurrent episodes and 50% of CD episodes (we did not use it in UC episodes) (p= 0.02).

At the moment of recurrent CDI, 21% of episodes were being treated with biological treatment. It was more frequent (75%) in CD than in UC (10%) (p=0.00). Specifically Adalimumab was the most used in 75% of CD episodes too (p=0.00). Infliximab and Adalimumab were both used in 5% of UC episodes.

Apheresis used in 21% of recurrent episodes. 1 of 4 episodes in UC treated with it. We did not use it in the treatment of CD episodes.

Immunosuppressant treatments combinations: 75% of episodes treated with at least an immunosuppressive drug+/-biological treatment+/-apheresis but 1 out of the four episodes (25%) were not treated, as well as 30% of episodes in UC and 0% of CD (1 out of 4 patients did not have immunosuppressor treatment). However in recurrent episodes of CD at least one of these drugs were

used. 42% of recurrent episodes treated with one drug and 33% of recurrent episodes with two drugs. Neither episode treated with a combination of 3 treatments. No differences regarding the combination treatment between UC and CD.

Table. IBD treatment at the moment of recurrent CDI

	Recurrent episodes (n=24)	**Ulcerative colitis (n=20)**	**Crohn´s disease (n=4)**	**p p=0.00**
Oral mesalamine	22 (92%)	20 (100%)	2 (50%)	P=0.02
Rectal mesalamine	9 (38%)	9 (45%)	0%	NS
Immunosuppressant				p=0.01
treatment	16 (67%)	13 (65%)	3 (75%)	NS
Azathioprine	11 (46%)	10 (50%)	1 (25%)	
Mercaptopurine				NS
Methotrexate	3 (13%)	3 (15%)	0%	
	2 (8%)	0%	2 (50%)	p=0.02
Biological treatment	5 (21%)	2 (10%)	3 (75%)	p=0.00
Infliximab	1 (4%)	1 (5%)	0%	NS
Adalimumab	4 (17%)	1 (5%)	3 (75%)	p=0.00
Apheresis treatment	5 (21%)	5 (25%)	0	NS
Treatment combinations (IS+/-biological+/-apheresis)	18 (75%)			NS
0	6 (25%)	6 (30%)	0%	
1	10 (42%)	8 (40%)	2 (50%)	
2	8 (33%)	6 (30%)	2 (50%)	
3	0%	0%	0%	

Risk factors

25% (1 out of 4) of recurrent episodes were treated with antibiotics three months before (metronidazole 100%; it is a CD-antibiotic). 71% of episodes had PPIs consumption three months before. Less than 10% episodes had episodes of hospitalization three months before, and neither episode presented surgery three months before. We did not find significant differences when we compared the results between UC and CD.

70% of recurrent episodes treated with PPIs without differences between UC and CD. Less than 10% of recurrent episodes had a personal history of hospitalization three months before the episodes, and 0% of patients had had surgery three months before these episodes (without differences between UC and CD).

	Recurrent episodes (n=24)	**Ulcerative colitis (n=20)**	**Crohn´s disease (n=4)**	**p value**
Antibiotics 3 months before **Metronidazole**	6 (25%) 6 (25%)	6 (30%) 6 (100%)	0%	NS
PPIs 3 months before	17 (71%)	13 (65%)	4 (100%)	NS
Hospitalization 3 months before	2 (8.3%)	1 (5%)	1 (25%)	NS
Surgery 3 months before	0%	0	0	NS

4.4.3 Independent risk factors for recurrent CDI episodes

Logistic regression analysis

Variables en la ecuación

		B	Error estándar	Wald	gl	Sig.	Exp(B)	95% C.I. para EXP(B)	
								Inferior	Superior
Paso 1[a]	TtolS(1)	1,457	,480	9,208	1	,002	4,294	1,675	11,006
	Constante	,754	,303	6,182	1	,013	2,125		
Paso 2[b]	tipoenf(1)	1,484	,613	5,859	1	,015	4,413	1,326	14,680
	TtolS(1)	1,680	,503	11,146	1	,001	5,368	2,001	14,396
	Constante	,234	,360	,424	1	,515	1,264		
Paso 3[c]	tipoenf(1)	2,286	,812	7,925	1	,005	9,831	2,002	48,267
	TtolS(1)	1,753	,522	11,282	1	,001	5,769	2,075	16,041
	ttobiolo(1)	2,692	,962	7,827	1	,005	14,761	2,239	97,305
	Constante	-2,373	1,036	5,243	1	,022	,093		

a. Variables especificadas en el paso 1: TtolS.

b. Variables especificadas en el paso 2: tipoenf.

c. Variables especificadas en el paso 3: ttobiolo.

We performed the multivariate analysis with the variables with statistical signication or $p<0.2$ in univariate analysis (CI 95%).

Risk profile of IBD patients with CDI recurrences episodes (independent factors):

- Patients with ulcerative colitis
- Immunosuppressants treatment
- Biological treatment.

Laboratory parameters in recurrent episodes

The interpretation of our results in the laboratory parameters was limited because of the small size samples. It was because physicians do not always ask for laboratory parameters for all their patients in the relapses. The average values were not different between UC and CD. Correlations bivariate positives ($p<0.05$) ferritin and CRP ($r=0.8$) and with significance ($P<0.01$) Fe and albumin ($r=1$), Fe and calprotectin ($r=1$), calprotectin and albumin ($r=1$). Negative correlations ($p<0.01$): ferritin and albumin ($r= -0.9$).

We did not find differences between severity and levels of laboratory parameters in recurrent episodes.

	Recurrent episodes (n=24)	**Ulcerative colitis (n=20)**	**Crohn´s disease (n=4)**	**p value**
Calprotectin	N=6 (25%)	N=4 (20%)	N=2 (50%)	NS
Mean	1864.83±2437.200	890.75±581.578	3813.00±4159.202	
0-200				
200-500	1	1	0	
>500	5			
		3	2	
	0%	0	0	
CRP	N=11	N=7	N=4	NS
	2.6845±4.52821	1.5486±2.71346	4.6725±6.73373	
ESR	N=12	N=8	N=4	NS
	18.83±10.803	18.13±12.017	20.25±9.323	
Albumin	N=5	N=3	N=2	NS
	3.6600±0.60663	3.7333±0.64291	3.5500±0.77782	
Leucocytes	N=13	N=9	N=4	NS
	9900.00±6503.204	9577.78±7593.711	10625.00±3792.427	
Platelets	N=13	N=9	N=4	NS
	290307.69±78818.763	283333.33±78310.280	306000.00±89565.618	

Hemoglobin	N=13 13.2846±1.17604	N=9 13.5444±1.29239	N=4 12.7000±0.64807	NS
Fe	N=4 58.5000±4.52821	N=3 62.6667±32.86842	N=1 46.000	NS
Ferritin	N=9 38.8778±46.36261	N=8 42.3000±48.33322	N=1 11.5000	NS

Correlaciones

		Hemoglobina	Ferritina	Hierro	PCR	VSG	Albumina	Plaquetas	Leucocitos	Calprotectina fecal
Hemoglobina	Correlación de Pearson	1	-,651	-,725	-,169	-,126	,685	,195	,239	-,809
	Sig. (bilateral)		,057	,275	,620	,695	,202	,523	,431	,097
	Suma de cuadrados y productos vectoriales	16,597	-305,042	-72,100	-8,294	-18,367	2,410	216961,538	21960,000	-9973,660
	Covarianza	1,383	-38,130	-24,033	-,829	-1,670	,603	18080,128	1830,000	-2493,415
	N	13	9	4	11	12	5	13	13	5
Ferritina	Correlación de Pearson	-,651	1	,917	,853*	-,290	-,995**	-,245	-,275	-,975
	Sig. (bilateral)	,057		,083	,015	,486	,005	,526	,473	,143
	Suma de cuadrados y productos vectoriales	-305,042	17195,936	3230,250	684,135	-1125,325	-98,565	-7237077,778	-789412,222	-8539,900
	Covarianza	-38,130	2149,492	1076,750	114,022	-160,761	-32,855	-904634,722	-98676,528	-4269,950
	N	9	9	4	7	8	4	9	9	3
Hierro	Correlación de Pearson	-,725	,917	1	,643	-,682	1,000**	-,508	-,632	1,000**
	Sig. (bilateral)	,275	,083		,357	,318	.	,492	,368	.
	Suma de cuadrados y productos vectoriales	-72,100	3230,250	2369,000	38,855	-625,000	,550	-4238000,000	-608550,000	11884,500
	Covarianza	-24,033	1076,750	789,667	12,952	-208,333	,550	-1412666,667	-202850,000	11884,500
	N	4	4	4	4	4	2	4	4	2
PCR	Correlación de Pearson	-,169	,853*	,643	1	,153	-,595	,535	-,198	-,209
	Sig. (bilateral)	,620	,015	,357		,673	,405	,090	,559	,791
	Suma de cuadrados y productos vectoriales	-8,294	684,135	38,855	205,047	61,430	-3,612	200810,909	-63853,091	-12814,927
	Covarianza	-,829	114,022	12,952	20,505	6,826	-1,204	200061,091	-6385,309	-4271,642
	N	11	7	4	11	10	4	11	11	4
VSG	Correlación de Pearson	-,126	-,290	-,682	,153	1	-,196	,484	,319	,323
	Sig. (bilateral)	,695	,486	,318	,673		,752	,111	,312	,596
	Suma de cuadrados y productos vectoriales	-18,367	-1125,325	-625,000	61,430	1283,667	-4,100	4707166,667	252433,333	22803,600
	Covarianza	-1,670	-160,761	-208,333	6,826	116,697	-1,025	427924,242	22948,485	5700,900
	N	12	8	4	10	12	5	12	12	5
Albumina	Correlación de Pearson	,685	-,995**	1,000**	-,595	-,196	1	,307	,401	1,000**
	Sig. (bilateral)	,202	,005	.	,405	,752		,616	,503	.
	Suma de cuadrados y productos vectoriales	2,410	-98,565	,550	-3,612	-4,100	1,472	38180,000	8526,000	108,400
	Covarianza	,603	-32,855	,550	-1,204	-1,025	,368	9545,000	2131,500	108,400
	N	5	4	2	4	5	5	5	5	2
Plaquetas	Correlación de Pearson	,195	-,245	-,508	,535	,484	,307	1	,311	-,221
	Sig. (bilateral)	,523	,526	,492	,090	,111	,616		,301	,721
	Suma de cuadrados y productos vectoriales	216961,538	-7237077,778	-4238000,000	200810,909	4707166,667	38180,000	7,455E+10	1914600000	-262967800
	Covarianza	18080,128	-904634,722	-1412666,667	200061,091	427924,242	9545,000	6212397436	159550000,0	-65741950,0
	N	13	9	4	11	12	5	13	13	5
Leucocitos	Correlación de Pearson	,239	-,275	-,632	-,198	,319	,401	,311	1	-,364
	Sig. (bilateral)	,431	,473	,368	,559	,312	,503	,301		,547
	Suma de cuadrados y productos vectoriales	21960,000	-789412,222	-608550,000	-63853,091	252433,333	8526,000	1914600000	507500000,0	-38748900,0
	Covarianza	1830,000	-98676,528	-202850,000	-6385,309	22948,485	2131,500	159550000,0	42291666,67	-9687225,000
	N	13	9	4	11	12	5	13	13	5
Calprotectina fecal	Correlación de Pearson	-,809	-,975	1,000**	-,209	,323	1,000**	-,221	-,364	1
	Sig. (bilateral)	,097	,143	.	,791	,596	.	,721	,547	
	Suma de cuadrados y productos vectoriales	-9973,660	-8539,900	11884,500	-12814,927	22803,600	108,400	-262967800	-38748900,0	29699720,83
	Covarianza	-2493,415	-4269,950	11884,500	-4271,642	5700,900	108,400	-65741950,0	-9687225,000	5939944,167
	N	5	3	2	4	5	2	5	5	6

*. La correlación es significativa en el nivel 0,05 (2 colas).

**. La correlación es significativa en el nivel 0,01 (2 colas).

Treatment of CDI recurrent episodes

95% of recurrent episodes (first, second and third) were treated in the same way, with oral metronidazole. We used oral vancomycin in less than 5% of recurrent episodes. Moreover, we did not use spiraxin or fidaxomicin. We used probiotics in less than 5% of episodes.

88% of recurrent episodes were treated with corticosteroids: 90% with prednisone, mean doses 29 mg (with differences between UC and CD, we used higher doses in CD (p=0.00)

	Recurrent episodes (n=24)	Ulcerative colitis (n=20)	Crohn´s disease (n=4)	p value
Oral metronidazole	23 (96%)	19(95%)	4(100%)	NS
Oral vancomycin	1 (4%)	1(5%)	0%	NS
IV metronidazole	0%			
Spiraxin	0%			
VSL3	1 (4%)			
Fidaxomicin	No			
		1(5%)	0%	
Corticosteroids	21(88%)	17(85%)	4 (100%)	NS
Prednisone	16 (67%)	12 (60%)	4 (100%)	
Beclomethasone	5 (21%)	5 (25%)	0	
Mean doses (mg)	29.25±17.417	25.31+/-17.173	45+/-5.774	p=0.00

Outcome in recurrent episodes

We found in the study of recurrent episodes that in 38% of episodes, therapeutic escalation was necessary 6 months after. Hospitalization was necessary 6 months after 17% of episodes. In both cases, there were no differences between CD and UC. We did not find colectomies 1 year after of episodes. Our patients did not have complications, neither mortality, because of CDI per se.

	Recurrent episodes (n=24)	Ulcerative colitis (n=20)	Crohn´s disease (n=4)	p
Therapeutic escalation 6 months after	9 (38%)	8 (40%)	1 (25%)	NS
Hospitalization 6 months after	4 (17%)	3 (15%)	1 (25%)	NS
Surgery 1 year after (colectomy)	0%	0%	0%	
Complications	0%	0%	0%	
Mortality	0%	0%	0%	

Chapter 4

5. Chapter 4: Discussion of results

In order to facilitate reading, this section will follow a similar structure as the results section before. In particular, we have divided this discussion into the following parts: epidemiological aspects, general characteristics of IBD, risk factors for acquisition of CD in IBD, outcome, recurrent CDI and CDI treatment. Finally, we will conclude by pointing out the major contributions and several limitations of our dissertation, as well as our proposals for future research.

Epidemiological aspects

The incidence of CDI is increasing worldwide, and it has become a relevant health concern for the international community. Its treatment is a challenge in the USA and the UK, where it has produced multiple and severe outbreaks. It can be a serious disease that can compromise the patients' lives. Proof of all this is the recent creation of the first faecal bank in the USA for the treatment of more severe and recurrent CDI episodes.

In Spain, the overall situation with respect of CDI is not well-known at the moment. There are a few epidemiological studies on the prevalence of CDI in the general population, and the available data indicate that CDI incidence is within the range of surrounding countries but not increasing.(188)

IBD patients have a higher incidence of CD in comparison with the general population. (31, 32) IBD is one of the strongest comorbidities associated with the possibility of CDI.(30) The prevalence of CDI in IBD patients in Spain is not well characterized and, only recently, one study published about Spanish IBD patients, just evaluated specific risk factors.(189) Outbreaks have not been reported in Spanish IBD patients in contrast with the general population, but with very few cases compared with other European cities and North America. (188, 190)

In our tertiary teaching center, we have not found an increase in the number of positive episodes in both, general population and IBD patients, during the period of the study from June 2007 to June 2015. Moreover, the number of positives CDI episodes exhibited a constant o decreasing tend, never increasing in the last 8 years. Thus, the situation in IBD patients in our area does not seem to be the same compared with the general population. There has been an increase in awareness about the existence of CDI and its influence on inflammatory bowel disease patients. In the USA, administrative database studies have suggested that the incidence of CDI on IBD, as in the general population, was increasing, but their design makes it hard to distinguish the real scope of the study with a consequent detection bias. (43) There is an apparent steady increase in CDI incidence complicated IBD in the last 10 years, specifically in UC and colonic Crohn´s disease.(39, 43)This increased incidence is more evident in large

studies as compared with smaller ones, but it is very hard to assess because of the differences between the used diagnosis methods.(42)

We have found that the proportion of positive results was low when compared with the number of requested samples. Several reasons could explain it: one explanation may be that in our centre, physicians requested control samples for most of the patients after their treatment. The latest guidelines to manage CDI (152) in the general population (there is not specific guidelines about management of CDI in IBD patients) recommend that after treatment samples are requested if the patient did not have a good evolution or the patient did not respond to the treatment or reinfection is suspected. If the patient improves after the treatment, it is not demonstrated that a routine request is necessary to confirm the response to treatment. Furthermore, one request sample is enough for CDI diagnosis. In addition, in most cases, we asked for an early CDI study in all the relapses. We should not routine request a study of stools in all IBD relapses from the start of the process. We should request it when the patients have risk factors for the infection, do not respond to the optimization of basal treatment, corticosteroids treatment, intensification of treatment and in hospitalized patients. (1)

In our Gastroenterology Department, CDI diagnosis took an average of 14 days, but the positive result was coming out of the Microbiology Department in an average time of 2 days. Therefore, there is a remaining gap of 10-12 days in which it would be possible to know the sample results. We could improve the early diagnosis of CDI in IBD outpatients with a better communication with the Microbiology Department. The result of the samples took between 24-48 hours to upload on our intranet. Moreover, another problem is the time that the patient delayed going back to the outpatient consultant. Thus, it would be helpful to keep in touch with the Microbiology Department in a more direct way to know the positive results as soon as possible.

General characteristics of IBD

Our study was performed in a single centre with a limited number of patients. However, it is the first large study about CDI in Spanish IBD patients and the first to evaluate recurrence and outcome in these patients. In our study, IBD patients with CDI were younger than the general population with CDI (>65 were less than 10%). CDI was community-acquired, and the study for CDI diagnosis was requested as an outpatient. Most studies published show that the age in IBD cohorts was much lower than in the general population controls suggesting that patients with IBD would have different risk profile. (42) Thus, our results are consistent with the literature

Most IBD patients appear to contract CD as outpatient even in hospitalized patients. If CDI confirms within 48 hours of admission, suggests a community acquisition.(39, 42, 43) In our study,

most analyzed CDI episodes were community acquisition without differences with non-CDI patients with a relapse. We could not find differences in the appearance of the infection in autumn and winter. This was probably because the Canary Islands have no seasons, with mostly homogeneous temperatures between 18-25ºC all the year round.

We found that UC and the rectal localization had more risk for CDI, and moreover UC was an independent risk factor for the CDI development in the multivariate analysis.(30, 47) Crohn´s disease with colonic involvement had a higher risk than small intestine localization for CDI. (43, 104, 105, 107) In addition, in our study, ileal localization was significantly more frequent in the control group. Our results are consistent with the literature; we found only in one study that a more extensive disease seemed to be at greater risk factor than a distal one. (43) In Crohn´s disease, non-stricturing non penetrating behavior was more frequent in CDI episodes than the stricturing one. We found in our serie that CDI was frequent in CD with perianal location and abscesses in the episodes, probably related to concomitant or previous antibiotic treatment. Thus, our findings suggest we should have a high index of suspicion for CDI in IBD patients mainly if they have a colonic involvement (ulcerative colitis and Crohn´s disease with colonic involvement), in CD with an inflammatory behavior and with perianal localization and an abscess in that episode.

There were more episodes of CDI and relapses than without CDI, during the first years of our follow-up. In fact, CDI episodes are frequent during the first 3 -7 years (50% and >70% respectively) after IBD diagnosis. It is known that IBD per se is a risk factor for CDI and could be because of the disease has not a completed control of inflammation, and this lack of inflammation control could be a predisposing factor. We do not know whether CD is a cause of IBD or a consequence of the inflammatory state in the intestinal environment and disruption of the normal microbiota. (18) However, the behavior of UC and CD with and without CDI was different: CDI makes the behavior of ulcerative colitis similar to the behavior of Crohn´s disease without CDI. Moreover, UC with CDI seems to have a different behavior as compared with UC without infection. UC with the infection has more probability of relapse and earlier than UC without infection, where the evolution only depends on its natural evolution. In contrast, in CD with the infection the relapses were less frequent when compared with CD without infection, in which the relapse depends on the natural evolution of the disease, without an external element triggering the relapse. Our results are interesting because CDI seems to play a role in the relapses in UC. There are questions unanswered about the role that CD can have in the exacerbations of IBD. (27)

Furthermore, in our study we found in more than 10% of the cases, a simultaneous CDI and IBD diagnosis compared with established IBD in the controls. It was an independent risk factor for CDI in IBD patients in the multivariate analysis. Our result is consistent with the literature and is important

because could support the role that CD can play in the onset of IBD. The prevalence of CDI in newly diagnosed IBD patients is high (8-10%) and is independent of the type of disease.(33, 34)

IBD episodes with CDI did not usually need hospitalization in our series, but we have found that these episodes had as risk factors treatment with PPIs 3 months before the episodes (similar to outpatients) and antibiotics in the limit of signification. Thus, it would be necessary try to avoid once the PPIs in this patients. The presence of comorbidities were not frequent in IBD patients, but patients with comorbidities had more risk for CDI. In fact, to have comorbidities was an independent risk factor in the multivariate logistic regression for CDI in our study. Nguyen et al.(104) found that the comorbidity increases the risk of acquisition of CDI in IBD patients.

We could not identify inflammatory markers that could be predictive factors for CDI development and predictive factors of a complicated CDI. Perhaps, due to our small sample size. In the general population there are laboratory markers of severity but no in IBD patients until now. We found more inflammation (higher levels of CRP) correlated with low levels of hemoglobin and higher levels of inflammation with low nutrition parameters. Albumin<3 gr (hyponutrition) was presented in both groups without differences, but there was a higher proportion in CDI group. Our study tried for the first time investigate the role of fecal calprotectin in CDI in IBD patients. However, we could not obtain significant differences with control group because of small sample size. However, calprotectin levels showed a tendency to be higher in CDI episodes (>500). Moreover, we found a tendency to higher calprotectin levels and clinical severity in our samples: in mild episodes the values had a tendency of being < 200, in moderate episodes, they had a tendency of being >500 and in the severe episode we did not have any samples to evaluate. In our opinion, faecal calprotectin could play a role in the classification of these patients in risk groups and in their follow-up. We need more studies to evaluate the role of this biological marker in CDI management.

Risk factors for acquisition of CD in IBD

IBD patients have a different risk profile as compared with the general population. One of the advantages of the database and other large studies is that their sample size has allowed the identification of risk factors associated with CDI. Most important pharmacological risk factors for CDI in the general population are the utilization of broad spectrum antibiotics (destroy the protective intestinal flora) and older age (associated with decreased immune response and increased comorbid conditions). However, it is common that CDI in IBD patients occurs in younger people, without antibiotics exposure and prior healthcare contact. The utilization of antibiotics in IBD is less than in general population, 43% CDI are associated with taking antibiotics 3 months before it.(72) In our study, antibiotics were used in 16% of CDI episodes as compared with IBD patients without CDI and the differences were significant (in the

control group, its use was more frequent in CD). Moreover, as in the case of comorbidities, it is less frequent compared with the general population but it is important in the CDI development in IBD patients. In our study antibiotic treatment 3 months prior episodes was an independent risk factor in the multivariate analysis. A stepwise antibiotic treatment in IBD patients would be essential.

In our study, PPIs were used significantly more in CDI group than in control group in the univariate analysis, and they were an independent risk factor at the limit of the multivariate logistic regression (but in our population there is an overuse of PPIs, which could be a confusion factor). Antiacids as risk factor for CDI have negative and positive reports. It is thought that the mechanism is related to decrease gastric acid, which increases the transit of the vegetative cells and spores of Clostridium beyond the stomach and cause infection. It is thought that proton pump inhibitors (PPIs) are more important risk factors than other antisecretory agents. In IBD patients, the use of PPIs have been evaluated in a few studies and the number of patients was too small to detect significant effects (35, 111). Recently, in 2015 Ramos-Martínez et al. (189) studied for the first time in Spain, risk factors in IBD patients for CDI and they found that PPIs treatment was a risk factor for CDI more than antibiotics. They also detected differences in spite of the small sample size.

We did not find differences in the use of immunosuppressants at the moment of episodes (40-50% in both groups) between CDI group and control group. Less than 10% of episodes with/without CDI were treated with biological treatment (infliximab and adalimumab) without differences. However, in the subgroup of recurrent episodes of CDI, immunosuppressant and biological treatment were risk factors in univariate and multivariate analysis. In the cohorts studies performed in a single centre is not frequent to be reported as a risk factor, perhaps because most were conducted before the widespread use of immunosuppressant treatment and were underpowered to detect small effect sized. On the contrary, Issa et al. (43) found that immunosuppression defined as the use of thiopurines, methotrexate, and steroids, double the risk of CDI in IBD patients. Schneeweiss et al. (191) did not find an association with infliximab or immunomodulators and the admission of IBD patient with CDI. Moreover, Seicean et al. (29) have suggested in several reported cases that infliximab treatment may be protective. We need more studies to confirm this and to stablish which would be the optimal infliximab treatment regimen for UC with CDI.

Outcome

In IBD population, the outcome has been different depending on whether the studies were carry out in a single centre, hospitalized patients or with data collected from a database. In our centre, we found a favorable outcome that confirmed the recent and only so far, Spanish study performed by Martínez et al. (189) in a single centre too. Overall, IBD patients with a relapse with CDI did not have

a worse outcome as far as therapeutic escalation and hospitalization 6 months after the episodes or surgery 1 year after the episodes are concerned. However, UC had a tendency for therapeutic escalation and hospitalization 6 months after the episodes, almost double than CD in CDI patients. However, CD had a tendency for more therapeutic escalation and hospitalization 6 months after episodes, double than UC in non-CDI patients. We had 2% of complications in Crohn´s disease patients because of perforations and 1 death in UC patients because of colorectal cancer, but unrelated to CDI per se. There are several studies in the USA which CDI associated with a negative impact clinical outcome, with an increased morbidity and mortality (39, 43, 105). Kelsen et al. (31) found that IBD patients with CDI required more hospitalization, escalation therapeutic and presented an increased severity.

There is an apparently adverse outcome associated to the combined use of antibiotics and immunosuppressant treatment compared with antibiotics alone, but we need prospective studies of CDI treatment in IBD patients specifically. In our study, immunosuppressant and biological treatment were independent risk factors for recurrences. However, they were not a risk factor in the first episodes. We did not find a worse outcome in IBD patients with CDI episodes who were being treated with immunosuppressant and biological treatment. Therapeutic escalation and hospitalization 6 months after the episodes, surgeries 1 year after them, complications and mortality, were not more frequent in CDI patients. Ben-Horin et al. (111) in a retrospective cohort study from European centres found that the use of one or more than one immunomodulators increased the risk of having an adverse outcome, independently of the severity of the disease at presentation (OR 17; 95% CI 3.2-91).

Furthermore, UC episodes, unlike CD, were treated with less immunosuppressant and biological treatments as compared with CD, but after CDI, UC needed more therapeutic escalation and CD more hospitalization (1 out of 4 episodes) but no statistically significant. We did not find differences between average hospital stay in both groups. In the literature, we found contradictory data related to the length of the hospital stay (LOS). In studies using databases, the LOS was significantly longer in the IBD patients than in controls. In contrast, reports from a single centre suggest that the LOS of IBD patients with CDI is similar to or shorter than controls.(42)

In our study, we did not have colectomies in UC patients 1 year after CDI and neither in the control group. We did not find colectomies in recurrent episodes 1 year after CDI. Variable rates of colectomies have been reported in IBD complicated by CDI. In a study in UC patients with CDI treated properly, reported a 44% of colectomies compared with 25% in non-CDI UC in a control group. (192) Issa et al. (43) reported in 2004 (45%) and 2005 (25%). Thus, in the USA the colectomy rates are much higher than the recent rates reported from Europe by Bossuyt et al. (58) 5% and Ben-Horin et al. (111) 6%. The differences between the colectomy rates depend on whether the patient´s CDI was treated in a centre with gastroenterologists interested in IBD and CD. It is likely that the index of suspicion for the diagnosis and use of empirical treatment of CDI would be better in these specialised centres.

Furthermore, the higher rate of colectomies in the USA were reported at the same time as hypervirulent strain NAP1/027 was endemic in the USA, and this could have contributed to the colectomies rate were higher in the USA than in Europe at the time.

Laboratory parameters used in European and American guidelines to define patients at risk of a complicated CDI were: older age, leucocytosis, renal failure and comorbidities. With regard to mortality, it was associated with age, comorbidities, hypoalbumin, leucocytosis, acute renal failure, and infection with ribotype 027. Most of IBD patients with CDI were young, without comorbidities or leucocytosis or hypoalbuminemia or renal failure. We did not serotype CD in stool samples. Thus, we did not find any laboratory parameter in CDI episodes associated to morbidity and mortality. There exist different results regarding mortality in the literature. There are database studies in hospitalized patients that suggest that 4% patients with CDI complicating IBD die on that admission. We had one death (0.7%) in an older patient with a colon cancer no directly related to CDI. The risk factors for mortality are increasing with: age, comorbidity, treatment in a teaching hospital, intestinal surgery and lack of an insurance policy. However, in a systematic review in 2011(42) analyzing the deaths in each of the included cohort studies they found only 1% (3/797) of patients had died, a similar prevalence that we observed in our study. This data are similar to mortality rate for IBD reported in the database studies. The database studies include all-cause mortality on that admission whereas the deaths reported in the single centres were more likely to be Clostridium difficile-related. Furthermore, Ananthakrishnan et al.(105) found that when the mortality rates were re-analyzed after removing the cases where IBD was listed as a secondary diagnosis, the association between mortality and IBD was no longer statistically significant.

Recurrent CDI

A few number of studies have evaluated the risk factors for recurrent CDI in adults IBD patients. It is estimated that 15-20% of IBD patients are going to have a recurrence of CDI. Goodhand et al. (42) in a meta-analysis in 2011 did not find relevant studies in adults with IBD, and there are few studies to evaluate specifically risk factors and outcome. Until now, our study is the first one that evaluates recurrent CDI episodes in Spanish IBD patients. We found that 18% of patients had some recurrent CDI episode (it is consistent with the literature). Moreover, after a first recurrence, second and third ones were frequent, too.

In our study, the episodes in UC had more risk compared with the ones in Crohn´s disease. Furthermore, more than one recurrent episode were frequent in UC (no more than one recurrence occurred in Crohn´s disease). We found that recurrent episodes were more frequent >8 weeks after the previous one except the second recurrence that was earlier. It may indicate that the antibiotic treatment

used was effective to cure CDI at that moment, but it was not effective to prevent new infections. However, the fact that recurrences occurred >8 weeks after the previous one could imply that new risk factors could play a role in addition to IBD per se. We did not genotype the CD strains so we cannot say if the new recurrences were of the same strain or a re-infection for another one.

We found that only 10% of episodes were in patients with age > 65 but in this subgroup, the ratio of recurrence was about 30%. Only 1 out of 4 episodes had been treated with antibiotics 3 months before, (but they were CD-antibiotics), and more than 70% of episodes were treated with PPIs 3 months before them. More than 1 comorbidity presented 20% of episodes and severity was more frequent mild-moderate (less than 5% of episodes were severe). Thus, it seems that the predisposing factors for CDI are partially overlapping in IBD patients as compared with general population. However, more than 80% occurred in outpatients, and 100% were community acquired, similar to CDI episodes in IBD patients either it is the first episode or not, unlike in the general population.

Garey et al. (114) published a meta-analysis to assess risk factors for recurrent CDI, in the general population. It was significantly associated with increased risk of recurrent CDI: continued use of non-CD antibiotics after CDI diagnosis, acid medications, and older age. In 2014, Abou et al. (123) performed another meta-analysis for recurrent CDI in non-IBD patients. These authors found that the risk factors for recurrences were: older age, use of antibiotics after diagnosis, PPIs and strain type. In 2015, Deshpande et al. (122) found that multiple risk factors are associated with the development of recurrent CDI. In the general population, prognostic markers used to determine risk of recurrent CDI (ESCMID) (152) are: age (>65), continued use of non-CDI antibiotics after diagnosis of CDI and/or after CDI treatment (Grade A), comorbidity (severe underlying disease) and/or renal failure (Grade A), a history of previous CDI (more than one recurrence) (Grade A), concomitant use of PPIs (Grade B) and initial severity (Grade B).

In our study, we found the type of disease (UC), immunosuppressant and biological treatment to be independent risk factors for recurrences. CDI episodes treated with combined treatment were 75%: immunosuppressant and/or biological treatment and/or apheresis. Moreover, UC episodes unlike CD, were treated with less immunosuppressant and biological treatment compared with CD but after recurrences, UC needed more therapeutic escalation and CD more hospitalization (1 out of 4 episodes). We did not find colectomies 1 year after or complications or death because of CDI. It would be important to perform prospective multicentre studies to have a larger sample size and statistical power.

Subgroup >65 years old

We think that is a subgroup with very interesting behavior to comment although we do not have previous data in the literature. The subgroup of > 65 years old was less than 10% of CDI episodes. They did not have comorbidities and diagnosis was done as outpatients unlike the general population with this age.

IBD had the same behavior as compared with younger patients: more frequent in UC and EC with colonic involvement. IBD diagnosis was done over 40 years old, and the infection occurred the first 5 years of IBD evolution. We found that 20% of episodes occurred at IBD diagnosis, and 50% needed hospitalization.

PPIs were frequent in CDI but no antibiotics. The ratio of recurrence was high (30%) which could justify that this subgroup was treated with antibiotics with a fewer rate of recurrences. At the moment of episodes, there was a high percentage of immunosuppressants and biological treatments (40% and 30% respectively) and 1 out of 3 patients had a combined treatment. They had a tendency to the escalation of treatment. Low percentages of episodes needed hospitalization, surgery and had complications and mortality in CDI group. This subgroup is small and larger studies are needed to confirm these results.

CDI treatment

We also need prospective, controlled trials to assess the best therapeutic approach in IBD patients with CDI. Even more, we need prospective and multicentres studies in IBD patients (because of its low prevalence) to define risk groups based on clinical severity and laboratory parameters and to accordingly evaluate the best therapeutic strategies. There are two essential aspects of the management of these patients: prophylaxis and early detection of CDI.

In our opinion, the first step in the management of UC with CDI should be to optimize oral and rectal treatment with mesalamine and antibiotic treatment. In our study, only 1 out of 3 patients was treated with rectal mesalamine at the moment of CDI episodes. The first step would be to optimize patients' base treatment adding rectal treatment and increasing the dose of oral mesalamine. A patient can have a relapse if he does not follow the treatment (in the case of distal UC if he does not use the local treatment). Often, optimizing the treatment is enough to control the relapse and requesting stool samples to investigate the presence of CDI is not needed. Thus, it would be crucial that physicians explained to the patients the importance of the adhesion to local treatment.

We used the same antibiotic treatment (oral metronidazole) in most episodes (one or more than one): mild-moderate or severe (although most of them were mild-moderate). However, we had 18% of recurrences. Moreover, we combined antibiotic treatment with corticosteroids in more than 70% of patients. Specifically prednisone was the most used in 1 out of 3 patients with average doses of 40 mg.

One of the main goals of IBD treatment is to avoid corticosteroids concomitantly. Thus, it would be beneficial for the IBD patient with CDI to start with antibiotic treatment alone in mild episodes without risk of complications or risk of recurrences. However, first of all, we had to define in this patients the severity of episodes and until now, we do not have specific guidelines. Thus, antibiotic therapy alone for CDI occurring in IBD patients with a severe relapse cannot yet be recommended. In hospitalized patients with a moderate-severe episode, it remains reasonable to start the treatment with intravenous corticosteroids and metronidazole or vancomycin while waiting the results of stool samples. However, our decision-making can wait for the results of stool samples in outpatients with a mild-moderate episode, and we can start optimizing the initial treatment of the patient. Thus, we could not treat with corticosteroids: mild-moderate episodes and episodes without severity clinical or in the laboratory parameters. For all reasons, strategies for the management of CDI in IBD patient should be done according to the age of the patient, biological markers of severity, and underlying comorbidities.

However, which is the best antibiotic treatment? The treatment with metronidazole and vancomycin is effective in more than 88% of cases (42). Thus, most patients respond initially to antibiotic therapy but 10-40% of them can have a post-treatment recurrence of diarrhoea in association with a repeatedly positive stool test for CD toxin (recurrence). CDI is hard to treat, and the recurrence after a first episode is high to justify more specific treatment in selected cases.

In our opinion, the key point is not only the treatment of the episodes. Metronidazole and vancomycin are equal regarding effectiveness but in the prevention of recurrences it seems that vancomycin is better. Therefore, probably after the first or following in severe episodes and patients with a high risk of recurrence, we should use vancomycin as the first line. However, these recommendations are in the general population, not specifically in IBD patients. We need prospective and controlled multicenter studies to evaluate the best treatment option for each subgroup of our IBD patients based on the number, severity, and risk of recurrence of episodes.

The episodes in our patients were more frequent with mild and moderate severity, and we used in most CDI first and recurrent episodes, oral metronidazole as the first line of treatment (more than 90%). Vancomycin and probiotics were used in a lower percentage of them. Guidance of European Society of Clinical Microbiology and Infectious Disease (ESCMID) (152) in section of recommendation on oral antibiotic treatment of the first episode on non-severe CDI recommends: metronidazole treatment, 500 mg three times daily for 10 days (Grade A) as first option and vancomycin 125 mg four times daily, 10

days (Grade B). Moreover, in the last sentence, they mention a different treatment option: "stop inducing antibiotics and observe the clinical response for 48 hours" (Grade C) (*see Appendices: Table 4*). Thus, we treated them based on this Guideline (ESCMID) (152) in the general population with the same characteristics.

However, the last sentence in this section is very interesting because: if we could have the results in the first 24 hours it would be possible, first of all, to optimize IBD treatment while we wait for the stools samples results. This point is also important in our case because our patients were not using local treatment at the moment of episodes, and, on the other hand, we could avoid an overuse of oral corticosteroids. We used a combination of antibiotic treatment and corticosteroids in more than 70% of patients. Moreover, prednisone was the most used one with an average dose of 40 mg. Thus, it would be necessary to start as soon as possible with the specific treatment for the infection: and avoid the use of corticosteroids in the mild cases. However, we do not have specific prospective studies in IBD patients where this procedure has been studied.

We had a low rate of severe initial episodes, but we treated them in the same way as first mild-moderate episodes. The recommendation in the Guideline (ESCMID) (152) is using vancomycin, 125 mg four times daily for 10 days or vancomycin, 500 mg four times daily for 10 days as treatment of the first line because it has a higher cure rate than metronidazole in this situation (Grade A) (*see Appendices: Table 5*).

Our rate of recurrences was high, but it was consistent with the literature. We used the same treatment for them (one or more than one): with oral metronidazole in more than 90% of episodes. The Guideline (ESCMID) (152) recommends as treatment of the first recurrence: vancomycin, 125 mg four times daily for 10 days (Grade B) or fidaxomicin, 200 mg twice daily for 10 years (Grade B). And as treatment of multiple recurrent CDI (more than one relapse): vancomycin 125 mg four times daily for 10 days, followed by pulse regimen (125-500 mg/day every 2-3 days) for at least 3 weeks (Grade B) or vancomycin 125 mg four times daily for 10 days, followed by taper regimen: gradually decreasing the dose to 125 mg per day. This subgroup of patients needs to treat it to avoid more recurrences (*see Appendices: Table 6 and 7*).

We need a prognostic marker in IBD patients specifically to improve the treatment of CDI in these patients. If we studied with detail the recommendations by ESCMID, the prognostic markers they used were: age≥65 years, leucocyte count >$15x10^9$, albumin <30 g/L, rise in serum creatinine level (≥1.5 times the premorbid levels) and comorbidities (severe underlying disease and/or immunodeficiency) and in this section they considered inflammatory bowel disease per se as risk factor to develop a severe CDI (*see Appendices: Table 2*). Thus, if we follow the general guidelines, IBD per se is a prognostic

marker for developing a severe CDI. Thus, we need specific guidelines for the treatment of CDI in IBD patients.

There are few data, and not enough controlled trials about the efficacy of other treatments for CDI in IBD patients such as probiotics and faecal transplantation. Both are used in the general population; faecal transplantation in severe and recurrent CDI episodes but IBD patients it has not yet been investigated in CDI treatment. In our opinion, faecal transplantation seems an option to treat CDI but also IBD per se, but we need controlled studies.

Limitations and strengths of our study

Our study has the following limitations: it is a retrospective study developed in a single centre with a small sample size. We revised paper medical records, and it was difficult the extraction of some data on severity of the disease. Furthermore, laboratory parameters were not requested in all relapses by physicians.

Despite this limitation, our findings are in agreement with previous studies and highlight the most important variables to consider when assessing risk factors for first and recurrent CDI episodes. Moreover, our study is the first in evaluating more than risk factors in CDI in Spanish patients: recurrence and outcome. Our findings could have implications for the treatment and control CDI episodes in IBD patients in our area and can help to establish patients profile at risk for CDI in Spanish IBD patients.

Future research

We acknowledge that this is just a first step in the medical research on this subject. It would be crucial the creation of a multidisciplinary workgroup to perform European multicenter studies and create protocol for diagnosis, treatment and prevention management in IBD patients.

Chapter 5

6. Chapter 5: Conclusions

- We did not find an increase in the number of cases of CDI from June 2007 to June 2015 in our area. The frequency of CDI is not characterized in IBD patients in Spain. Because of its low prevalence, multicenter and prospective studies are needed to evaluate the incidence and prevalence of CDI in IBD patients in Spain.

- Direct and fluid communication with the Microbiological Department is crucial to get positive results faster to make the therapeutic decision as early as possible.

- We should not routine request a stool sample in all IBD relapses from the start of the process. We should request it when the patients had risk factors for the infection

- CDI was frequent during the first years of follow-up and at IBD diagnosis.

- The behavior of UC and CD with and without CDI was different: CDI becomes the behavior of ulcerative colitis similar to the behavior of Crohn´s disease without CDI. UC with the infection has more probability of relapse and earlier than UC without infection. Nevertheless, in CD with the infection the relapses were less frequent compared to CD without infection in which the relapses depend on the natural evolution of the disease.

- We must have a higher grade of suspicion:

    - ✓ In patients with active colonic IBD (ulcerative colitis and Crohn´s disease with colonic involvement). We have found CDI more frequent in ulcerative colitis with rectal localization. Ulcerative colitis is an independent risk factor for CDI. Moreover, in Crohn´s disease with an inflammatory behavior, perianal localization and an abscess in that episode.

    - ✓ At IBD diagnosis, there is more risk for Clostridium difficile infection. We have found it to be an independent risk factor for CDI. These results could support the role that CD can play in the onset of IBD.

    - ✓ After any CDI episode, IBD patients have a higher probability of recurrence, especially after the first 8 weeks after stopping treatment.

- Most CDI episodes occurred in young patients and were community-acquired. The diagnosis was done as an outpatient. Thus, IBD patients have a different risk profile compared with the general population, and IBD per se could play a role in CDI.

- Most CDI episodes were mild-moderate. We need to establish prognostic markers to determine the risk of developing a severe CDI with prospective and multicenter studies. ESCMID guideline for the general population, considers IBD per se, as a prognostic marker of severe CDI.

- Percentage of hospitalization was low, but CDI patients needed significantly more. Most episodes had a moderate severity. Thus, we need to improve the communication with the emergency room to hospitalize only the patients who need it.

- We have not found any inflammatory biomarker in blood or stool or other laboratory parameter to be able to predict the severity of the CDI.

- In our area, the subgroup of patients over 65 years old seemed to have a different behavior with a worse outcome, prognosis, and more recurrent episodes.

- We did not find that immunossupressant and biological treatments were a risk factors for CDI except in recurrent episodes

- In our study in Spanish IBD patients, the independent risk factors in the multivariate analysis for CDI were: ulcerative colitis, at the time of diagnosis of IBD, antibiotics 3 months before episodes and comorbidity. PPIs 3 months before episodes was a risk factor in the univariate analysis. Two of these risk factors are preventable ones (antibiotics and PPIs), another we can suspect it early (at IBD diagnosis), and comorbidity is not modifiable but can be improved.

- Recurrent CDI is a frequent event and the independent risk factors for its development were: ulcerative colitis, immunosuppressant, and biological treatment.

- CDI did not have any influence on the outcome of the IBD relapse: no more therapeutic escalation, no more need for hospitalizations and long stay at hospital, no more need for surgery and finally no more mortality.

Chapter 6

7. Chapter 6: Our proposals to modify the management of CDI

Our proposals to modify the management of CDI in IBD patients in our area, according to our study are:

Prevention strategies

- In our hospital, it would be recommended the creation of a multidisciplinary workgroup for the elaboration of CDI guidelines for diagnosis, treatment and prevention strategies. We think that the following departments should be included: Microbiology, Infectious Disease, Internal Medicine, Preventive Medicine and Gastroenterology Departments.

- Awareness campaigns for a rational use of PPIs and broad spectrum antibiotics in outpatients and hospitalized patients. Both, are risk factors for CDI in our area.

- Preventive Department has done for a long time an impressive work on preventive strategies (contact isolation, hand washing, single rooms) to avoid CDI transmission in hospitalized patients.

- Elaboration of a specific protocol with Microbiology department could be useful to serotype CD in the stools samples of patients affected by the infection. We do not know if the hypervirulent ribotype 027 is or not in our hospital.

Diagnosis of CDI in our IBD patients

In general, we must have a higher grade of suspicion of CDI in IBD patients:

- Early suspicion in patients with a risk profile in our area. Thus, we can start the specific treatment as soon as possible: Ulcerative colitis; patients with comorbidities, taking PPIs and antibiotics and at the time of diagnosis of IBD.

- Direct and fluid communication with the Microbiological Department is crucial to get positive results faster to make the therapeutic decision as early as possible.

- We should not routine request a study of stools in all IBD relapses from the start of the process. We should solicitate it when the patients have risk factors for the infection or do not respond to the

optimization of basal treatment, corticosteroids, intensification of treatment and in hospitalized patients.

- One stool sample is enough to diagnose CDI except if we suspect a reinfection.

- It is not necessary to confirm the microbiological resolution with control stool samples after treatment if the patients have improved.

Treatment of CDI in our IBD patients

- One of the most relevant aspects of management of CDI is to prevent recurrences. Specifically, the treatment of the recurrent episodes and the patients who are at risk for recurrent CDI is a hard challenge.

- In our area, we have a high percentage of recurrences after a first episode. Thus, we should use specific antibiotics that were effective to treat the infection and prevent recurrences at the same time.

- How should we treat IBD patients with CDI in an episode? Is the same UC and CD? We are going to suggest the following treatment in our area based on our results, waiting for specific guidelines in Europe:

    - ✓ The first episode of non-severe CDI in outpatients: We could wait for results of stool samples, optimize the basal treatment of patients and start antibiotic treatment if positive result. We could begin with metronidazole (500 mg three times daily 10 days as the first option) in general in UC and CD but if we had other risk factors (antibiotics, PPIs, comorbidities, at IBD diagnosis) and UC, we think that we could start with vancomycin (125 mg four times daily 10 days) for the risk of recurrence.

    - ✓ Treatment for the first recurrence: we should use oral vancomycin, 125 mg four times daily 10 days

    - ✓ Treatment of severe CDI and hospitalized patients: we should use oral vancomycin, 125 mg four times daily 10 days

- ✓ Multiple recurrent episodes (more than one relapse): we should use oral vancomycin, 125 mg four times daily 10 days followed by pulse regimen (125-500 mg/day every 2-3 days) for at least 3 weeks.

- ✓ We found a high percentage of recurrence in patients over 65 years old, we should start the treatment with vancomycin, 125 mg four times daily 10 days for the risk of recurrence

- One of the main goals of IBD treatment is to avoid corticosteroids. We could not treat with corticosteroids the mild-moderate episodes (episodes without severity clinical or in the laboratory parameters). Therefore, it would be beneficial for the patient to start with antibiotic treatment alone in mild episodes without risk of complications or recurrences. We need prospective, controlled trials of CDI treatment in IBD patients. In hospitalized patients, it seems reasonable to start with intravenous corticosteroids and metronidazole or vancomycin while awaiting the results of stool samples. In outpatients in better condition, decision-making could await the result of stool testing.

- We need to elaborate specific protocols for treatment of CDI in IBD patients specifically. Thus, probably after a first recurrence, severe episodes and patients with a higher risk of recurrences, we should use vancomycin as the first line: in our area, UC recurs more than CD. Therefore, in UC treatment we should use vancomycin at least after the first recurrence. Moreover, should we treat with vancomycin all CDI in UC from first episodes or only in a subgroup of patients such as hospitalized one or at risk for recurrence or with severe episodes?

- We need prospective and multicentre studies in IBD patients to define risk groups with a worse evolution and evaluate the best strategies for them based on the age of the patient, biological markers of severity, and underlying comorbidities.

- We need prospective and controlled multicenter studies to evaluate the best treatment option for each subgroup of our IBD patients based on the risk of recurrence, the number of episodes and severity.

- The results of our study can be helpful to define risk groups for CDI. Additional or new interventions may be required to prevent the episodes (appropriate treatment/ avoid or prevent risk factors).

In our opinion, it would be necessary to create a multidisciplinary workgroup to develop guidelines for the management of this infection in IBD patients: diagnosis, treatment and prevention strategies.

Future research

Our proposals are:

- Prospective and multicentre studies in IBD patients to define risk groups with a worse evolution and evaluate the best strategies for them based on the age of the patient, biological markers of severity, and underlying comorbidities.

- Prospective and controlled multicenter studies to evaluate the best treatment option for each subgroup of our IBD patients based on the risk of recurrence, the number of episodes and their severity.

- Prospective studies of CDI treatment specifically in IBD patients: use of combined antibiotics and immunosuppressant treatment compared with antibiotics alone.

- Clinical studies are required to compare vancomycin and fidaxomicin for reducing recurrent CDI.

- Investigate the differences in risk profiles between IBD patients and the general population in Spain.

- It would be interesting to investigate the role that calprotectin could play in CDI in IBD patients.

- Study the role of vitamin D supplementation to prevent CDI with randomized and controlled trials

We need to work together; it would be necessary to create a multidisciplinary workgroup to study the best management of CDI and elaborate specific protocols about the best diagnosis methods, specific risk factors and specific treatments. This study is a first step in the study of CDI in Spanish IBD patients. We need to performance multicenter, prospective studies to evaluate the specific risk factors in IBD.

Appendices

8. Appendices

Table 1: Clinical features CDI

(Extracted from: European Society of Clinical Microbiology and Infectious Disease: update of the treatment guidance document for Clostridium difficile infection; 2014)

Sign/symptom	*Definition*
Diarrhoea	Loose stools, i.e. taking the shape of the receptacle or corresponding to Bristol stool chart types 5-7, plus a stool frecuency of three stools in 24 or fewer consecutive hours or more frequently than is normal for the individual.
Ileus	Signs of severely disturbed bowel function such as vomiting and absence of stool with radiological signs of bowel distension
Toxic megacolon	Radiological signs of distension of the colon (>6 cm in transverse width of colon) and signs of severe systemic inflammatory response.

Table 2: Prognostic markers we can use to determine the risk of developing a severe CDI

(Extracted from: European Society of Clinical Microbiology and Infectious Disease: update of the treatment guidance document for Clostridium difficile infection; 2014)

Characteristics	SoR [a]	QoE	Comment(s)
Age (≥65 years)	A	IIr	Large cohort study on CDI mortality at 30 days, and review of studies of factors associated with CDI outcome [41]. Systematic review of studies describing the derivation or validation of Clinical Prediction Rules for unfavourable outcomes of CDI [46]: in general methodological biases and weak validities.
Marked leucocytosis (leucocyte count > $15x10^9$ /L)	A	IIrht	Systematic review [46]: in general methodological biases and weak validities. Cohort study: severity score on malignancy, white blood cell count, blood albumin, and creatinine [37]. Retrospective cohort study on risk factors for severe CDI: death <30 days, ICU, colectomy or intestinal perforation [32].
Decreased blood albumin (<30 g/L)	A	IIr	Systematic review [46]: in general methodological biases and weak validities.
Rise in serum creatinine level (≥133 µM or ≥1,5 times the premorbid level)	A	IIht	Depending on the timing of measurement around CDI diagnosis [45]
Comorbidity (severe underlying disease and/or inmunodeficiency)	B	IIht	Comorbidity wide variety of risk factors described/investigated, including cancer, cognitive impairment, cardiovascular, respiratory and kidney disease [41]. Chronic pulmonary disease, chronic renal disease and diabetes mellitus [66]. History of malignancy [37]. Previous operative therapy, inflammatory bowel disease and intravenous immunoglobulin treatment [63]

[A] *soR: degree of recommendation to use a (clinical) characteristic as a prognostic marker*

Table 3: Prognostic markers we can use to determine risk of recurrent CDI

(Extracted from: European Society of Clinical Microbiology and Infectious Disease: update of the treatment guidance document for Clostridium difficile infection; 2014)

Characteristics	SoR [a]	QoE	Comment(s)
Age (>65 years)	A	IIrh	Meta-analysis: [43]. Systematic review [46]. Prospective validation study of risk factors: [42].
Continued use of (non-CDI) antibiotics after diagnosis of CDI and/or after CDI treatment	A	IIrh	Meta-analysis: [43] Prospective validation study of risk factors: [42].
Comorbidity (severe underlying disease) and/or renal failure	A	IIh	Prospective validation study of risk factors: comorbidity conditions rated by Horns´ index (scoring system for underlying disease severity) [42].
A history of previous CDI (more than one recurrence)	A	IIt	Data from randomized controlled trials [26,70] Meta-analysis of pivotal randomized controlled trials [40]
Concomitant use of antiacid medications (proton pump inhibitors)	B	IIrh	Meta-analysis on recurrent CDI [43]. Meta-analysis on CDI [72]
Initial disease severity	B	IIth	Prospective validation study of risk factors: [42]. Long-term population based cohort study [67]

[A]*soR: degree of recommendation to use a (clinical) characteristic as a prognostic marker*

Table 4: Recommendations on oral AB treatment of first episode of non-severe CDI

(Extracted from: European Society of Clinical Microbiology and Infectious Disease: update of the treatment guidance document for Clostridium difficile infection; 2014)

Treatment	SoR	QoE	Commnent(s)
Metronidazole, 500 mg three times daily 10 days	A	I	No statistically significant difference in cure rate between metronidazole and vancomycin or teicoplanin Statistically significant difference in sustained clinical cure between metronidazole and vancomycin in favour of vancomycin in one study (and pooled results of two randomized controlled trials published only in abstract form
Vancomycin, 125 mg four times daily 10 days	B	I	Cochrane analysis: teicoplanin significantly better than vancomycin for bacteriological cure and borderline superior in terms of symptomatic cure
Fidaxomicin, 200 mg twice daily 10 days	B	I	Evidence limited to two Phase III studies. Fewer recurrences as compared to vancomycin, except C. difficile CRP ribotype 027
Vancomycin, 500 mg four times daily 10 days	C	I	Vancomycin: Equal cure rate 500 mg four times daily orally compared with 125 mg four times daily orally.
Stop inducins antibiotic(s) and observe the clinical response for 48h	C	II	Rate of spontaneous resolution unknown in mild CDI. Studies performed before increased incidence of hypervirulent strains.

Table 5: Recommendations on oral antibiotic treatment of initial severe CDI

(Extracted from: European Society of Clinical Microbiology and Infectious Disease: update of the treatment guidance document for Clostridium difficile infection; 2014)

Treatment	SoR	QoE	Commnent(s)
Vancomycin, 125 mg four times daily for 10 days	A	I	Cure rate higher as compared with metronidazole in severe CDI
Vancomycin 500 mg four times daily for 10 days	B	III (Ia)	Randomized controlled trial on dose effectiveness: no significant differences in measurable responses of high-dose compared to low-dose regimens. However: results not stratified for severity of illness
Fidaxomicin, 200 mg twice daily 10 days	B	I	Evidence limited to two Phase III studies [70,91]. Fewer recurrences compared with vancomycin 125 mg four times daily in severe disease (except for CRP ribotype 027). No data on the efficacy in severe life-threatening disease and/or toxic megacolon: excluded from both studies.
Metronidazole, 500 mg three times daily for 10 days	D	I	Differences in symptomatic cure of metronidazole versus vancomycin not statistically significant in a pooled analysis [2]. ICU admission and hypoalbuminaemia (= disease severity) predictors of metronidazole failure

Table 6: Recommendations on oral antibiotic treatment

For mild/moderate first episode of CDI but with risk for recurrence or recommendation for first recurrence

(Extracted from: European Society of Clinical Microbiology and Infectious Disease: update of the treatment guidance document for Clostridium difficile infection; 2014)

Treatment	SoR	QoE	Commnent(s)
Vancomycin, 125 mg four times daily for 10 days	B	I	No statistically significant difference in recurrence rate between vancomycin and teicoplanin
Fidaxomicin, 200 mg twice daily for 10 days	B	I	Evidence limited to two Phase III Studies Retrospective subset analysis: fewer secondary recurrences with fidaxomicin (n = 16/79 patients) as compared with vancomycin (n = 26/80 patients) after treatment of a first recurrence Fidaxomicin was not associated with fewer recurrences in CDI due to CRP ribotype 027 as opposed to non-027.
Metronidazole, 500 mg three times daily for 10 days	C	I	Recurrence rate: metronidazole not inferior to vancomycin for treatment of mild primary CDI Or after a first recurrence. Vancomycin significantly more effective in bacteriological cure than metronidazole in recurrent CDI.
Vancomycin, 500 mg four times daily for 10 days	C	III	One randomized controlled trial on dose effectiveness in primary CDI: no significant differences in responses of high-dose compared with low-dose regimens vancomycin. However, results not stratified for recurrent CDI

Table 7: Recommendations on oral antibiotic

For treatment of multiple recurrent CDI (more than one relapse)

(Extracted from: European Society of Clinical Microbiology and Infectious Disease: update of the treatment guidance document for Clostridium difficile infection; 2014)

Treatment	SoR	QoE	Commnent(s)
Vancomycin, 125 mg four times daily for 10 days, followed by pulse regimen (125–500 mg/day every 2–3 days) for at least 3 weeks.	B	IIt	Retrospective case cohort of two placebo/antibiotic trials [Observational study: Expert opinion
Vancomycin, 125 mg four times daily for 10 days, followed by taper regimen: gradually decreasing the dose to 125 mg per day.	B	IIt	] Retrospective case cohort of two placebo/antibiotic trials. Observational study:. Expert opinion
Fidaxomicin, 200 mg twice daily for 10 days	B	IIrt	Evidence limited to two Phase III studies. Retrospective subset analysis: fewer recurrences as compared to vancomycin treatment after first recurrence. Systematic review. Efficacy after multiple recurrences was not investigated
Vancomycin, 500 mg four times daily for 10 days	C	IIrt	Retrospective case cohort of two placebo/antibiotic trials. Trend for lower recurrence frequency for high-dose vancomycin. Systematic review
Metronidazole, 500 mg three times daily for 10 days	D	IIrt	Retrospective case cohort of two placebo/antibiotic trials.]. Trend for lower recurrence frequency for high-dose vancomycin and low-dose metronidazole. Systematic review

Table 8: Recommendations on non-antibiotic treatment

In combination with antibiotic treatment or recurrent CDI (more than one relapse)

(Extracted from: European Society of Clinical Microbiology and Infectious Disease: update of the treatment guidance document for Clostridium difficile infection; 2014)

Type of intervention	Treatment	SoR	QoE	Commnent(s)
Faecal or bacterial instillation	Vancomycin, 500 mg four times daily, 4 days + bowel lavage + nasoduodenal infusion donor faeces	A	I	Also many observational studies and meta-analyses.
Probiotics	Vancomycin or metronidazole + Saccharomyces boulardii	D	I	Comparison of relapse rates: in subgroup analysis efficacy in recurrent CDI, but not in initial CDI. Evidence-based review
	Vancomycin or metronidazole + Lactobacillus spp	D	I	Evidence-based review
Passive immunotherapy with immune whey	Colostral immune whey	D	I	Study interrupted early

Table 9: Recommendations on non-oral antibiotic treatment of initial CDI: mild and severe disease

(Extracted from: European Society of Clinical Microbiology and Infectious Disease: update of the treatment guidance document for Clostridium difficile infection; 2014)

Patient subgroup	Treatment	SoR	QoE	Commnent(s)
Non-severe disease.	Intravenous metronidazole 500 mg three times daily for 10 days	A	IIu	Retrospective uncontrolled study
Severe disease and/or complicated or refractory CDI	Intravenous metronidazole 500 mg three times daily for 10 days +	A	IIru	Retrospective uncontrolled study
	vancomycin retention enema 500 mg in 100 mL normal saline four times daily intracolonic for 10 days	B	III	Systematic review Expert opinion
	Intravenous metronidazole 500 mg three times daily for 10 days +	A	IIru	Retrospective uncontrolled study
	vancomycin 500 mg in 100 mL normal saline four times daily by oral/nasogastric tube for 10 days	B	III	Retrospective uncontrolled study. Systematic review. Expert opinion
	Intravenous tigecycline 50 mg twice daily for 14 day	C	III	Observational study/case report

Table 10: Recommendations on alternative treatments for first episode of CDI

(Extracted from: European Society of Clinical Microbiology and Infectious Disease: update of the treatment guidance document for Clostridium difficile infection; 2014.

Type of intervention	Treatment	SoR	QoE	Commnent(s)
Immunotherapy	Human monoclonal antibodies against TcdA and TcdB with standard oral antimicrobial therapy (metronidazole and vancomycin)	C	I	Evidence limited to Phase II randomized controlled trial. Primary endpoint changed during study. Reduced recurrence of CDI: analysis for recurrence only performed in those who were cured, received >7 days of antimicrobial therapy and did not receive intravenous gammaglobulins
	Passive immunotherapy with immune whey after standard oral antimicrobial therapy	C	II	Observational study: 101 CDI patients (40% recurrent CDI). Results suggest reduction in recurrence rate.
Probiotics	Oral vancomycin or oral metronidazole + Saccharomyces boulardii	D	I	Comparison of relapse rates: in subgroup analysis efficacy in recurrent CDI, but not in initial CDI. Evidence-based review

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RESUMEN EN ESPAÑOL

10. Resumen en español

10.1 Abstract

Clostridium difficile (CD) es la causa más frecuente de diarrea en pacientes hospitalizados y de diarrea asociada a tratamiento antibiótico, sin embargo también puede aparecer en la comunidad. Además en las dos últimas décadas la prevalencia de la infección por Clostridium difficile ha aumentado en todo el mundo e incluso, han casos graves y resistentes al tratamiento convencional. Por otra parte se han producido brotes en pacientes hospitalizados en Europa, Estados Unidos y Canadá.

Los pacientes con enfermedad inflamatoria intestinal (EII), enfermedad de Crohn y colitis ulcerosa, son un grupo de alto riesgo para la infección por CD. Esta puede ser detectada tanto al diagnóstico inicial de la EII como durante los brotes, por lo que resulta de gran importancia mantener un alto índice de sospecha para iniciar un tratamiento específico precoz y reducir las complicaciones.

En España, sólo hay un estudio que evalúe los factores de riesgo para la infección por CD en los pacientes con EII. Sin embargo, necesitamos conocer la importancia y las implicaciones de esta infección en los pacientes con EII con un brote de actividad en nuestro país.

Este es el primer estudio que se realiza en las Islas Canarias sobre Clostridium difficile y pacientes con EII que añade a un estudio previo realizado en España la valoración, no sólo de los factores de riesgo, sino la recurrencia de la infección y la evolución en los pacientes.

Objetivos:

Los propósitos de este estudio fueron (1) describir los factores de riesgo en la infección por CD en los pacientes con EII, (2) analizar la recurrencia de la infección por CD en estos pacientes, (3) investigar la influencia de la infección por CD en la evolución de la EII y (4) establecer un perfil de riesgo en paciente de riesgo en los pacientes con EII.

Pacientes y métodos:

Pacientes:

Elaboramos un estudio caso-control retrospectivo en pacientes adultos (edad> 14 años) con un brote de EII. El estudio fue realizado por los Departamentos de Digestivo y Microbiología de un Hospital Universitario de tercer nivel en Las Palmas de Gran Canaria (España) durante el periodo comprendido entre junio de 2007 hasta junio de 2015.

Criterios de inclusión:

Los casos fueron definidos como pacientes de EII con un brote de la enfermedad (diarreas con deposiciones líquidas), con toxina para CD positiva en las muestras de heces. Los controles fueron pacientes con EII también en brote, pero con toxina para CD negativa en sus muestras de heces.

El criterio de exclusión para los controles fue infección por CD previamente conocida.

Métodos:

Tanto los casos como los controles fueron identificados en la base de datos del Laboratorio de Microbiología de nuestro hospital. Los controles se aleatorizaron mediante una aleatorización simple sin repeticiones con el programa Excel 2010 y emparejados 1:1 en el mismo periodo de tiempo con los casos. La infección por CD se diagnosticó mediante la detección de toxinas de CD en muestras de heces mediante ELISA desde 2007 a diciembre de 2012 y un test de tres pasos (GDH, toxina de CD y PCR) desde enero de 2013.

El resto de datos fueron recogidos de la historia clínica del paciente entre los que se incluyen: factores de riesgo epidemiológico, datos clínicos, características de la EII incluyendo: localización, tratamiento, cirugías previas hasta 3 meses antes del brote, ingreso hospitalario y datos analíticos (incluyendo calprotectina fecal). Además estudiamos la recurrencia y la evolución hasta 6 meses después de la infección (necesidad de colectomía, escalada terapéutica, hospitalización o fallecimiento).

El análisis de los datos fue realizado con el programa SPSS 22: el análisis univariante se realiza con Chi-cuadrado y t de student para la comparación de las variables cualitativas y cuantitativas respectivamente. Cuando fue necesario se utilizaron sus correspondientes test no paramétricos (Test exacto de Fisher y U-Mann Witney). Finalmente se realizó un análisis multivariante para identificar los factores de riesgo para la infección por CD.

10.2 Introducción general

La infección por Clostridium difficile (ICD) en los pacientes con enfermedad inflamatoria intestinal (EII) es un tema fascinante. Clostridium difficile (CD) es causa de diarrea en humanos desde hace más de 30 años y su incidencia está aumentando de forma alarmante en la última década. Desde principios del año 2000 graves epidemias por CD han ocurrido en pacientes hospitalizados, en Europa y Norteamérica y han puesto en jaque a las autoridades sanitarias. Incluso, ha llegado a convertirse en un grave problema de salud debido a sus altas tasas de complicaciones y mortalidad.

Los medios de comunicación han cubierto las noticias, muchas veces en primera página, sobre las epidemias más importantes de CD en Reino Unido y Norteamérica. Recientemente, en septiembre de 2015, se ha creado en Estados Unidos el primer banco de heces para el tratamiento de la ICD recurrente, lo cual proporciona una clara evidencia de la importancia de esta infección y de que existe un aumento del número de personas que contraen esta infección.

¿Qué es el Clostridium difficile y por qué resulta tan difícil su control y tratamiento?

Clostridium difficile (CD) es la causa más frecuente de diarrea en pacientes hospitalizados y de diarrea asociada a tratamiento antibiótico. Es una bacteria Gram positiva, anaerobia estricta y formadora de esporas. Las esporas se diseminan fácilmente por vía aérea y son capaces de sobrevivir en condiciones adversas durante largos periodos de tiempo. Las esporas pueden contaminar el medio hospitalario a través de los trabajadores y por prácticas de limpieza ambiental inadecuadas.

CD vive de forma inofensiva en el intestino de entre un 10 a un 15% de los adultos sin causar síntomas (colonización). Sin embargo, cuando aparece un disbalance bacteriano, a menudo a consecuencia de un tratamiento con antibióticos, puede producirse un amplio abanico de manifestaciones clínicas que abarcan desde portador asintomático, diarreas sin complicaciones

a infecciones graves que pueden comprometer la vida del paciente.

En la última década, la severidad y mortalidad de las infecciones se ha incrementado debido a la aparición de cepas más virulentas (ej. B1/NAP1/027), resistentes al tratamiento antibiótico convencional. Así, hemos tenido una gran cantidad de problemas con el tratamiento y la prevención de los nuevos casos de infección y de la recurrencia.

El tratamiento incluye medidas generales tales como de soporte y de control de la infección y el tratamiento antibiótico que debería iniciarse lo antes posible. El trasplante fecal podría constituir otra opción de tratamiento en los casos de ICD graves o recurrentes.

Qué pacientes están en riesgo para contraer una infección por Clostridium difficile?

Es bien conocido que el perfil típico del paciente con CD: hospitalizado, de edad avanzada con tratamiento antibiótico, con enfermedades crónicas e inmunodeprimidos. Sin embargo, el número de personas que contraen la infección fuera del entorno hospitalario está en aumento, la exposición al tratamiento antibiótico no resulta suficiente para producir la infección y por primera vez se describe un significativo incremento en la incidencia de CD en niños y embarazadas.

Un grupo de pacientes especialmente susceptible para la ICD son los pacientes con EII (enfermedad de Crohn y colitis ulcerosa) y particularmente aquellos con afectación colónica. A diferencia de los pacientes que no tienen una enfermedad inflamatoria intestinal (EII), los pacientes con EII y con ICD suelen ser más jóvenes, con menor exposición a tratamiento antibiótico y en la mayoría de los casos con adquisición en la comunidad. Por lo tanto, el paciente con EII tiene un perfil de riesgo completamente diferente a los de la población general

¿Cuál es la relación entre Clostridium difficile y la enfermedad inflamatoria intestinal? ¿Puede ser tan peligrosa como en la población general?

La relación entre CD y la EII es controvertida. Todavía no está claro si el CD es una causa etiológica o una consecuencia de la enfermedad inflamatoria. Se desconoce si el problema es por la alteración en el sistema inmune local en el intestino, el estado inflamatorio sistémico o ambos La infección por Clostridium difficile juega un importante papel en el inicio clínico de la EII, produce retrasos en el diagnóstico de nuevos casos y dificulta el diagnóstico diferencial en los brotes.

La infección por Clostridium difficile añade dificultades al manejo terapéutico de la EII, puesto que requiere un tratamiento antibiótico específico.

Varios estudios en la EII han demostrado un aumento en la gravedad y en la tasas de recurrencia de la enfermedad, ambas asociadas a un incremento de la morbilidad, la necesidad de cirugías e incluso de la mortalidad

¿La recurrencia de la infección por CD podría ser un problema para los pacientes con EII? ¿Cuál sería el mejor enfoque terapéutico?

La recurrencia puede llegar a suponer un problema importante después de un primer curso de tratamiento en pacientes con y sin EII, alrededor de un 30% en cada uno de ellos. En la EII se recomienda la detección del Clostridium en muestras de heces durante los brotes de reactivación de la enfermedad resistentes al tratamiento convencional en pacientes ambulatorios y en todos los pacientes, en base a las directrices de práctica clínica europea, americana y española.

Sin embargo, no existen recomendaciones específicas en los pacientes con EII para su tratamiento y manejo en general. Hoy en día, tratamos a nuestros pacientes según las Guías clínicas de práctica clínica de los Servicio de Microbiología y enfermedades infecciosas. Por

esta razón sería importante que tuviéramos protocolos específicos para un mejor manejo, tanto diagnóstico como terapéutico en los pacientes con EII.

Hemos diseñado un estudio retrospectivo, caso-control para evaluar los siguientes aspectos de la infección por CD en nuestra área:

¿Cómo ha cambiado el número de pacientes de EII con infección con Clostridium difficile en los últimos ocho años?

¿Cuáles son los factores de riesgo para la ICD en los pacientes con EII en nuestro medio?

¿Es la recurrencia más frecuente en nuestros pacientes? ¿Cómo los tratamos?

¿Cómo tratamos la infección por CD en nuestros pacientes con EII?

¿Cómo afecta la infección por CD la evolución de la EII?

Nuestro estudio es el primero que se realiza en las Islas Canarias acerca de la infección por CD en pacientes con EII, y el primero en España en añadir experiencia a los estudios de los factores de riesgo, el estudio de la recurrencia de la infección, el diagnóstico y la evolución de la misma. Necesitamos conocer las implicaciones de esta infección en los pacientes con EII en nuestro medio

10.3 Marco teórico

La enfermedad inflamatoria intestinal (EII) es una inflamación intestinal crónica cuya etiología y patogenia aún no están totalmente conocidos. En general, la enfermedad de Crohn (EC) y colitis ulcerosa (UC), son enfermedades poligénicas y multifactoriales. Se ha sugerido que varios factores ambientales, microbianos, inmunológicos, genéticos y de estilo de vida juegan un papel en su iniciación. En estudios realizados desde la década de 1980 hasta la actualidad, el Clostridium difficile (CD) se ha implicado como un factor de riesgo para la reactivación del proceso inflamatorio en hasta un 5% de los pacientes con EII.

Múltiples estudios en los últimos 10 años han señalado tasas más altas de ICD tanto colonización como infección por CD en pacientes con EII. La toxina de CD se detectó en los pacientes con enfermedad inflamatoria del intestino, especialmente en aquellos con recaídas sintomáticas. En algunos casos, no fue registrada la administración de antibióticos previa al brote, y los síntomas respondieron a la vancomicina. Anteriormente, algunos "recaídas" se habían producido por "actividad de la enfermedad" de la enfermedad inflamatoria del intestino subyacente.

Algunos científicos pensaban que algunos tratamientos médicos (por ejemplo, sulfasalazina) podrían alterar la flora intestinal y promover la colonización por CD. Otros teorizaron que el estado inmune alterado, posiblemente relacionado con agentes terapéuticos, o el estado nutricional podría desempeñar un papel importante.

Por lo tanto, los pacientes con EII son considerados un grupo de riesgo para la ICD, pero el riesgo de infección no puede ser completamente explicada por los factores de riesgo conocidos, en general. Las anormalidades en la respuesta inmune de la mucosa en la EII podrían desempeñar un papel en la ICD.

A finales de 1970 la detección de la toxina de Clostridium difficile durante los brotes de la EII no estaba recomendado. Pero en 2002 en un congreso de la Digestive Disease Week en EEUU , se describió por primera vez la presencia del CD y sus toxinas en un número significativo de pacientes con EII. La importancia de esta asociación aún no se conocía, pero en este congreso se comentó ya que las pruebas diagnósticas adecuadas podrían ayudar a asegurar que estos pacientes reciben el mejor tratamiento.

Los nuevos retos terapéuticos de este patógeno, han traído un renovado interés en todas las facetas de la enfermedad. En estos últimos años, las últimas guías de práctica clínica y documentos de consenso europeos, americanos y españoles recomiendan investigar CD en las reactivaciones de la EII. Entonces, ¿qué ha cambiado? ¿Es necesaria la investigación de rutina en todos los pacientes con EII con una recaída o sólo en algunos casos específicos?

El manejo de la infección por CD en pacientes con EII con una reactivación sintomática no ha sido optimizado. Por el momento, no disponemos de guías clínicas específicas para el manejo de la infección en los pacientes con EII. Este hecho resulta contradictorio, ya que mientras todas las Sociedades de EII (tanto la Europea como la Americana) recomiendan la investigación de CD en las muestras de heces de aquellos pacientes, tanto ambulatorios como hospitalizados, que tengan un brote grave resistente al tratamiento convencional, no existen recomendaciones específicas para el tratamiento de la infección en este tipo de pacientes. La elaboración de guías clínicas específicas en EII sería de gran importancia para mejorar el tratamiento de esta infección de una manera homogénea en España y Europa.

Por este motivo, debemos seguir las indicaciones de la Guía General de Microbiología para la población general En 2009 fue publicada la primera guía de tratamiento de la infección por CD de la Sociedad Europea de Microbiología Clínica y Enfermedades Infecciosas

(ESCMID) siendo de inmediato ampliamente aplicada a la practica clínica. La última Guía de la ESCMID ha sido publicada en 2014.

En particular, tras el reciente desarrollo de la fidaxomicina, nuevos fármacos alternativos para el tratamiento de la ICD en los EE.UU. y Europa, existe una creciente necesidad de información actualizada sobre la eficacia comparativa de los agentes antibióticos disponibles en la actualidad para el tratamiento de la ICD, proporcionando así las recomendaciones basadas en la evidencia sobre esta patología.

Las recomendaciones para mejorar la orientación clínica en el tratamiento de la ICD, se especifican para diferentes grupos de pacientes, tales como la enfermedad no grave, ICD severa, primera recurrencia o riesgo para la enfermedad recurrente, múltiples recurrencias y el tratamiento de la ICD cuando la administración oral no es posible.

La opciones terapeúticas incluyen: antibióticos, probióticos y trasplante intestinal fecal. Los antibióticos recomendados son: metronidazol, vancomicina, y fidaxomicina. El trasplante fecal está indicado para el tratamiento de recurrencias múltiples. A continuación vamos a revisar las opciones terapeuticas más importantes

10.4 Recomendaciones generales para la prevención y control de la ICD

En los países desarrollados, Clostridium difficile es la causa más común de infección adquirida en el hospital. La infección por CD es una causa frecuente de morbilidad e incluso de muerte. También produce enormes costes económicos, ya que los pacientes infectados por CD en el hospital prolongan su estancia durante unos 1-3 semanas adicionales. En cuanto a costes y la productividad, C. difficile es una carga importante para nuestro sistema de salud.

10.5 Puntos claves para la prevención y el control de la infección por CD

Desde nuestro punto de vista, hemos identificado tres puntos clave:

1-Control de los factores de riesgo.

Cuando usamos antibióticos es importante realizar una escalada terapéutica adecuada. Incluso si fuera posible tendríamos que suspender el tratamiento antibiótico en los pacientes con ICD. En la mayoría de los casos, no podemos eliminar el tratamiento con antibióticos por lo que debemos disminuir el uso de antibióticos de amplio espectro. Otro factor a tener en cuenta es la malnutrición del paciente, lo cual resulta ser un factor predisponente en centros de larga estancia.

2-Diagnostico precoz (alta sospecha diagnóstica)

Axelrad et al. estudió el uso de un protocolo de admisión de enfermería para aumentar la tasa de detección de ICD. La intervención incrementó el número de pruebas para la ICD en los pacientes hospitalizados por un brote de EII. La infección hay que sospecharla en pacientes

ambulatorios con una recaída resistente al tratamiento convencional o con recaídas y factores de riesgo para la infección. Por otra parte, sospecharemos una ICD en todo paciente hospitalizado que presente una recaída o empeore durante la hospitalización.

3- Medidas de higiene y profilaxis para la ICD:

Evitar la propagación por el personal médico a otros pacientes y la contaminación con esporas del medio hospitalario y las superficies evitando la transmisión horizontal. Los pacientes por lo general adquieren el organismo desde el hospital. Por desgracia, las esporas son difíciles de eliminar de las salas del hospital, y algunos hospitales han experimentado brotes de CD que continuaron durante años.

La transmisión paciente-paciente puede ser la más importante para el aumento del ratio de la infección por CD. La transmisión secundaria entre los pacientes se ve facilitada por las habitaciones estrechas, baños y salas de estar compartidas, y la socialización con otros pacientes. Además, es posible encontrar contaminación ambiental en habitaciones de no aislamiento, en las áreas de trabajo de médicos y enfermeras, y en el equipo portátil. Necesitamos otros estudios para determinar si la contaminación en esas áreas puede jugar algún papel en la transmisión del CD.

Por el contrario, Daneman et al. encontraron que las estrategias de prevención de hospitales seleccionados no produjeron una reducción estadísticamente significativa del riesgo para contraer una ICD. Estas estrategias tenían una eficacia limitada o se implementaron de manera ineficaz, al menos, durante el período del estudio.

Es importante señalar que los brotes de CD pueden ocurrir tambien en las residencias de ancianos. Por tanto, es necesario que los sistemas de salud y los de asistencia social trabajen en estrecha colaboración para proteger a las personas que tienen a su cuidado.

En resumen, los métodos más importantes de la prevención son: la administración de antibióticos, la higiene de manos y el aislamiento del paciente afectado por la infección. El aislamiento se mantendrá hasta 48 horas después de la resolución del cuadro entérico. Los pacientes afectados permanecerán en habitaciones separadas. Es importante insistir en la educación del personal sanitario, y en la intensificación de la limpieza del medio ambiente. Otras medidas de control tales como la comunicación, la educación, el refuerzo de las medidas de control de infecciones, la optimización del diagnóstico y tratamiento son importantes también.

Los investigadores de la Clínica Mayo recomiendan la práctica de la prevención, que incluyen:

- Lavarse las manos con agua y jabón.
- Limpiar las superficies contaminadas con lejía (1.000 pm)
- Evitar el contacto con personas que se sabe que tienen ICD.
- Tomar precauciones si vive con una persona que tiene una ICD o trabaja en un centro de salud donde puedan estar expuestos a pacientes con ICD.

Por último, nos gustaría destacar la importancia de una vigilancia continua de la ICD en los hospitales, sobre todo cuando un grupo de riesgo está expuesto, como los pacientes con EII.

Son necesarios en todos los hospitales campañas de vigilancia y prevención activa como "las campañas para el lavado de manos"

10.6 Estudio

10.6.1 Objetivos:

Los propósitos de este estudio fueron:

(1) describir los factores de riesgo en la infección por CD en los pacientes con EII

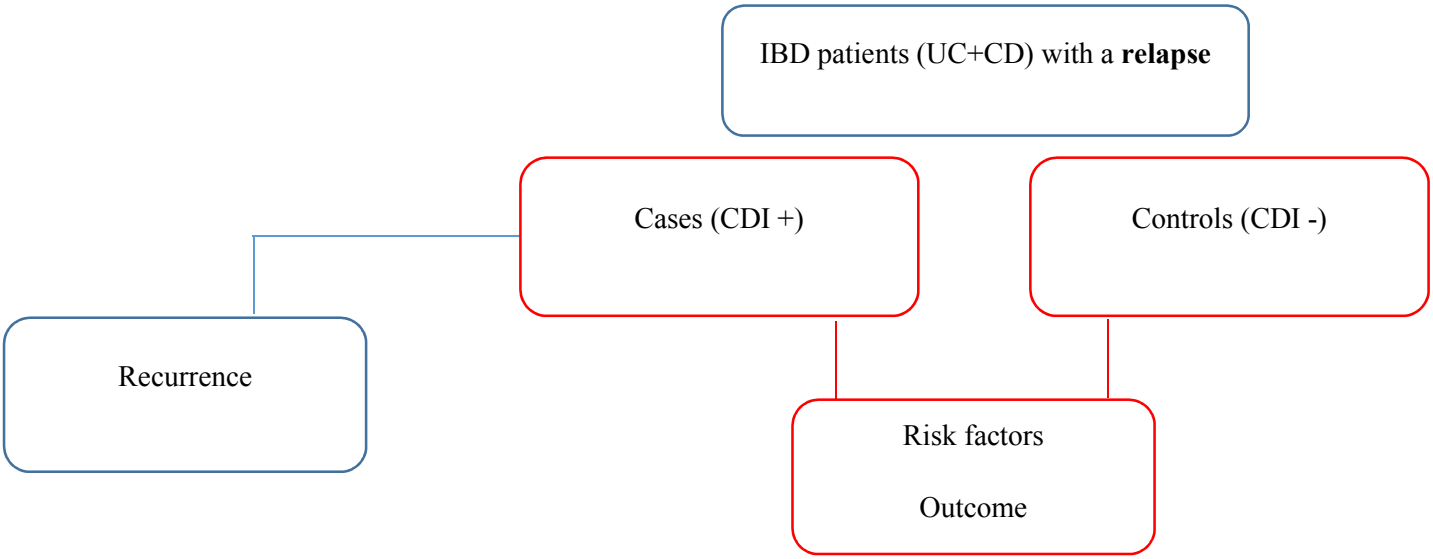
(2) analizar la recurrencia de la infección por CD en estos pacientes

(3) investigar la influencia de la infección por CD en la evolución de la EII

(4) establecer un perfil de riesgo para la infección por CD en nuestra área.

10.6.2 Metodología.

Gráfico del diseño del estudio



Pacientes

Elaboramos un estudio caso-control retrospectivo en pacientes adultos (edad> 14 años) con un brote de EII. El estudio fue realizado por los Departamentos de Digestivo y Microbiología de un Hospital Universitario de tercer nivel en Las Palmas de Gran Canaria (España) durante el periodo comprendido entre junio de 2007 hasta junio de 2015.

Criterios de inclusión: Los casos fueron definidos como pacientes de EII con un brote de la enfermedad (diarreas con deposiciones líquidas), con toxina para CD positiva en las muestras de heces. Los controles fueron pacientes con EII también en brote, pero con toxina para CD negativa en sus muestras de heces.

Episodio de infección por CD: cuadro clínico compatible con ICD (diarrea: tres o más deposiciones liquidas durante dos días o más) y la evidencia microbiológica de toxinas o la presencia de CD en las heces, sin evidencia razonable de otra causa de la diarrea.

ICD severa: se define como un episodio de ICD con (uno o más signos y síntomas específicos) colitis severa o un curso complicado de la enfermedad, lo que lleva a la necesidad de ingreso en una UCI, colectomía o la muerte.

Uno o más de los siguientes factores pronósticos desfavorables pueden estar presente sin evidencia de otra causa: marcada leucocitosis (> 15 10 9 / L), disminución de la albúmina en la sangre (<3 g / L) y aumento del nivel de creatinina sérica (> 1,5 veces los nivel premórbido).

Episodios recurrentes de ICD: la presencia nuevamente de los signos y síntomas de la ICD tras un periodo asintomático, junto con un análisis de heces positiva para CD.

Recurrencia: ICD <8 semanas después de la aparición de un episodio anterior, con síntomas que habían desaparecido y reaparecen tras la finalización del tratamiento previo.

Reinfección: ICD > 8 semanas después de la aparición de un episodio anterior, con síntomas que habían desaparecido y reaparecen tras la finalización del tratamiento previo.

El criterio de exclusión para los controles fue infección por CD previamente conocida

Métodos

Tanto los casos como los controles fueron identificados en la base de datos del Laboratorio de Microbiología de nuestro hospital. Los controles se aleatorizaron mediante una aleatorización simple sin repeticiones mediante el programa Excel 2010 y emparejado 1:1 en el mismo periodo de tiempo con los casos. La infección por CD se diagnosticó mediante la detección de toxinas de CD en muestras de heces mediante ELISA desde 2007 a diciembre de 2012 y un test de dos pasos (GDH, toxina de CD) y PCR desde enero de 2013.

El resto de datos fueron recogidos de la historia clínica del paciente entre los que se incluyeron: factores de riesgo epidemiológico, datos clínicos, características de la EII incluyendo localización, tratamiento, cirugías previas hasta 3 meses antes del brote, ingreso hospitalario y datos analíticos (incluyendo calprotectina fecal). Además estudiamos la recurrencia y la evolución hasta 6 meses después de la infección (necesidad de colectomía, escalada terapéutica, hospitalización o fallecimiento).

El análisis de los datos fue realizado con el programa SPSS 22. Las comparaciones estadísticas se realizaron con la t de Student (variables cuantitativas) y pruebas de X2 (variables cualitativas) y cuando fue necesario prueba U-Man W. y test exacto de Fisher (OR con IC del

95%). Se realizó un análisis de regresión logística con variables que resultaron significativas en el análisis univariado para identificar factores de riesgo de la ICD

10.6.3 Resultados

Entre junio de 2007 junio de 2015, se analizaron 131 episodios de ICD en pacientes con EII con una reactivación de la enfermedad (grupo de casos) en comparación con pacientes con una recaída, pero sin infección (grupo control).

Por otra parte, se estudiaron los episodios recurrentes de ICD por separado.

Vamos a mostrarles los resultados de nuestro estudio con el siguiente esquema:

- En primer lugar, nos gustaría dar una visión general de la distribución de la ICD (resultados positivos); una comparación de los resultados positivos en los pacientes con EII con los resultados positivos en los pacientes sin EII en el mismo período de tiempo y ver la relación entre la proporción de muestras positivas con respecto al total de muestras solicitadas.
- En segundo lugar, veremos cuánto tiempo se tarda en diagnosticar a estos pacientes en nuestro hospital.
- En tercer lugar, explicaremos los resultados del estudio de casos y controles, incluyendo los factores de riesgo.
- Y finalmente, comentaremos nuestros resultados del estudio de los casos recurrentes

Distribución de los episodios de ICD

En nuestro centro terciario, no hemos encontrado un aumento del número de episodios de ICD tanto en los pacientes con EII y sin EII durante el período del estudio. Por otra parte, el número de episodios positivos para la ICD tiende a mantenerse o disminuir, pero en ningún caso se ha incrementado en los últimos 8 años. Nos dimos cuenta de que la proporción de resultados positivos fue baja en comparación con el número de solicitud de muestras.

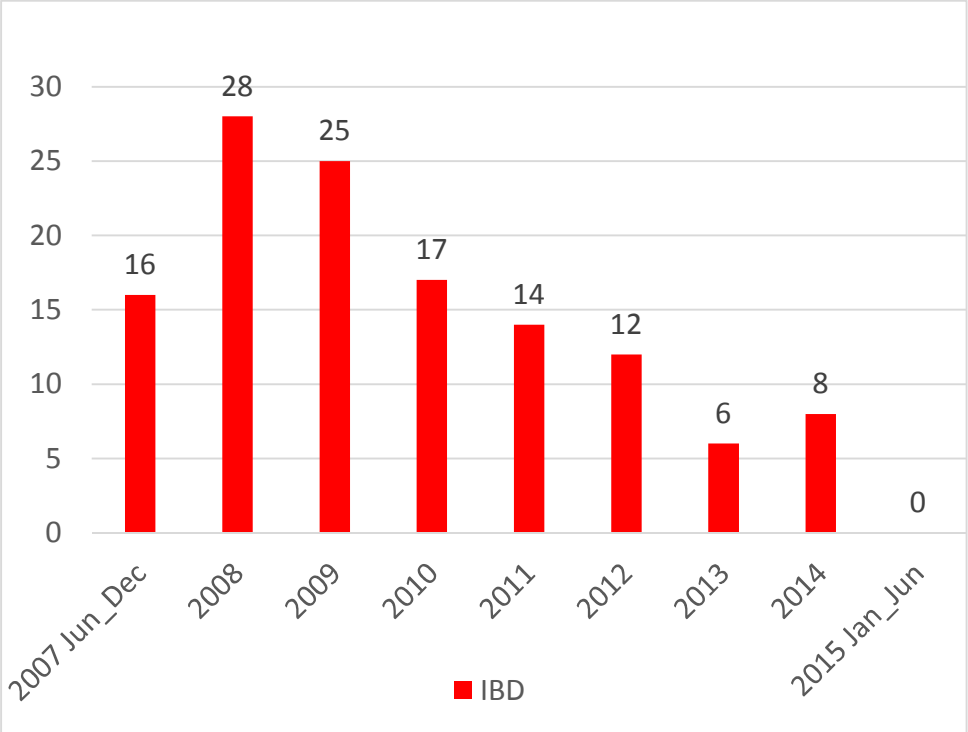


Figura. Distribución de los episodios positivos durante el periodo de estudio

En la distribución de las muestras positivas a partir de Junio de 2007 a Junio de 2015. Se puede observar que el número de episodios positivos han ido disminuyendo a lo largo del período de estudio. Así, desde Enero a Junio de 2015, no hemos tenido ningún caso de ICD.

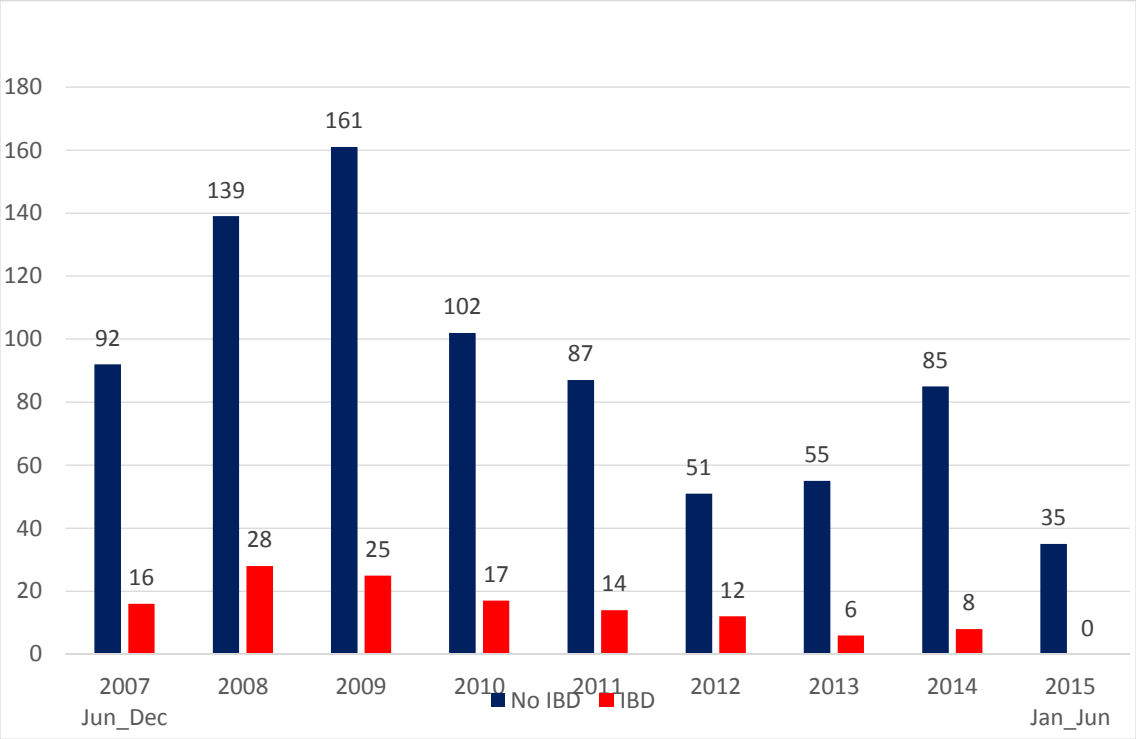


Figura. Distribución episodios positivos en comparación con la población general

Distribución episodios positivos en pacientes con EII en comparación con resultados positivos en población no-EII desde junio de 2007 hasta junio de 2015. La distribución de la ICD en la población general no se ha incrementado durante los últimos 6 años, pero al mismo tiempo, se ha mantenido en similares proporciones, en contraste con la población con EII

El número de muestras solicitadas es mayor que el número de resultados positivos de manera proporcional. Encontramos una baja proporción de resultados positivos en comparación con el gran número de solicitudes.

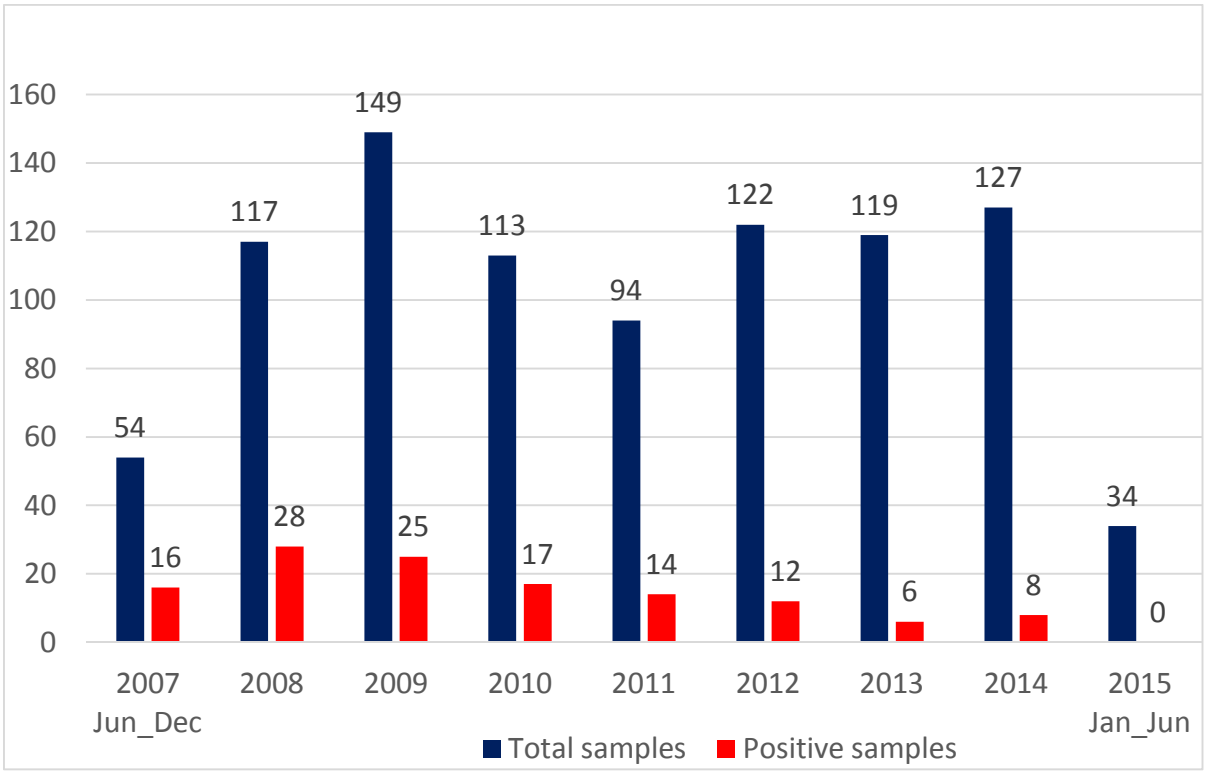


Figura: Total de muestras solicitadas en pacientes con EII en comparación con los resultados positivos finales**.**

Tiempo que se tardó para diagnosticar nuestros pacientes

El tiempo medio necesario para que los resultados positivos sean emitidos desde el departamento de Microbiología fue de 4,22 ± 6,281 días. El tiempo necesario desde la emisión del resultado positivo hasta que llega a conocimiento del médico fue de 7,59 ± 6,223 días y el tiempo que transcurre desde la primera cita hasta la entrega de los resultados al paciente fue 15.58 ± 8,265 días.

	Tiempo que tarda el resultado positivo en emitirse desde el departamento de Microbiología	**Tiempo hasta que el resultado llega al médico solicitante**	**Tiempo que transcurre desde la primera visita hasta la segunda**
Media(días)	4.22±6.281	7.59±6.223	15.58±8.265
Mediana	2.00	7.00	14.00
Modo	2	7	14
Minimo-maximo	1-53	0-30	4-42

Tabla: Días necesarios para diagnosticar la ICD en pacientes ambulatorios

En nuestro departamento, la infección por CD tuvo un promedio de 14 días para el diagnóstico, pero el resultado positivo se emitió por el Departamento de Microbiología en un

tiempo promedio de 2 días. Por lo tanto, hay un espacio de unos 10-12 días en los que sería posible saber los resultados de las muestras.

Puntos clave de los resultado en estudio caso-control

Aspectos epidemiológicos

- En nuestro centro terciario, no nos hemos encontrado un aumento en el número de episodios de CDI entre junio de 2007 y junio de 2015.

- Debemos mejorar el diagnóstico precoz de la ICD en nuestros pacientes ambulatorios con EII con una mejor comunicación con el Departamento de Microbiología. La infección por CD es una enfermedad prevenible y tratable.

- No solicitar estudio de CD desde el inicio de las recaídas, excepto cuando existen factores de riesgo específicos, refractariedad al tratamiento y hospitalización: En primer lugar, optimizar el tratamiento oral y el tratamiento rectal. Es importante preguntar al paciente por su adhesión al tratamiento tópico.

Características generales de los episodios

- Nuestros pacientes con EII con ICD fueron más jóvenes que la población general (> 65 menos del 10%).

- Hubo una mayor adquisición comunitaria y la solicitud del estudio se realizó como pacientes ambulatorios.

- En general, no hubo comorbilidad, pero había más comorbilidad asociada con las recurrencias. Fue un factor de riesgo en el estudio univariante y también un factor de riesgo independiente en el estudio multivariante

- En la mayor parte de los casos, los pacientes con EII afectados por una ICD no requirieron hospitalización.

- La severidad leve-moderada de los episodios fue más frecuente en los pacientes ambulatorios y también en los hospitalizados por un brote con ICD.

Características de la EII

- La edad media de inicio de síntomas de la EII (17-40 años)

- La infección es signicativamente más frecuente en los pacientes con CU y localización rectal en el estudio univariante y resulto ser un factor de riesgo independiente en el multivariado. En la EC la localización más frecuente de manera significativa fue la colónica y el comportamiento inflamatorio.

- ICD fue más frecuente en la EC con una localización perianal y con abscesos en el momento del episodio.

- El tiempo de evolución de la EII hasta la aparición de la infección fue de 7 años aproximadamente sin diferencias entre los dos grupos.

- La infección por Clostridium difficile hace que el comportamiento de la colitis ulcerosa sea similar al comportamiento de la enfermedad de Crohn sin infección por CD. Por lo tanto, la CU con una ICD tiene una tendencia a mas recidivas y más precoces. La tendencia a la recidiva en la CU es mayor en los casos y la de la EC en los controles. Sin embargo, sin la infección, la EC evolucionó peor que la UC.

- Los pacientes con EC sin infección tuvieron una mayor tendencia a las recidivas que cuando tuvieron la infección.

- Encontramos un porcentaje de un 20% de ICD en el momento del diagnóstico de la EII. Este resultó ser un factor de riesgo independiente para la adquisición de la ICD en nuestros medio en el estudio multivariante. Debemos sospechar una sobreinfección por CD en el momento del diagnóstico inicial de una EII, para iniciar el tratamiento específico tan pronto como sea posible. Es obligatorio, al diagnóstico de una EII, hacer un diagnóstico diferencial con otras causas de diarrea infecciosa, como la infección por Clostridium difficile.

Parámetros de laboratorio

- La calprotectina fecal tenía una tendencia a tener valores más altos en los episodios de CDI en comparación con los controles Necesitamos más estudios para ver el valor real de este marcador biológico en el manejo de la ICD.

- Se observaron correlaciones negativas significativas pero débiles entre la PCR y hemoglobina; y entre la VSG y albúmina.

- No encontramos parámetros de desnutrición (albúmina <3 gr/dl) con más frecuencia en el grupo de los pacientes con la infección.

Factores de riesgo para la ICD

- Encontramos que el tratamiento antibiótico 3 meses previo al episodio y con IBP fueron factores de riesgo en el análisis univariante y además el tratamiento antibiótico fue un factor de riesgo independiente en el análisis multivariante

- Los tratamientos inmunosupresores y biológicos no fueron un factor de riesgo para un primer episodio de CDI pero si fueron un factor de riesgo independiente para los episodios recurrentes. No se encontró más proporción de tratamiento combinado (inmunosupresor y fármacos biológicos).

- En pacientes hospitalizados con una ICD el tratamiento con IBP y/o antibiótico 3 meses antes fueron también factores de riesgo en el estudio univariado.

- Encontramos un bajo porcentaje de pacientes en tratamiento con mesalazina tópica en el momento de la infección.

Outcome

- Los pacientes con EII con un brote de su enfermedad relacionado con una infección por CD no tuvieron una evolución peor que los pacientes en recidiva pero sin ICD en cuanto a: escalada terapéutica y hospitalización 6 meses después del episodio o cirugía un año después. Sin embargo, la CU tuvo tendencia a una mayor escalada terapéutica y hospitalización 6 meses después de los episodios, casi el doble que la EC y en los pacientes con ICD. En cambio, la EC tuvo tendencia a la escalada terapéutica y hospitalización 6 meses después del episodio el doble que en la CU pero en pacientes sin ICD.

- Encontramos un 2% de complicaciones y 1 muerte en pacientes con EII y una ICD.

Tratamiento de la infección por Clostridium difficile

- La mayoría de los episodios se trataron tanto los primeros como los episodios recurrentes con metronidazol oral. La vancomicina, spiraxin y probióticos se utilizaron en unos pocos pacientes.

- Combinamos el tratamiento antibiótico con corticosteroides en 3 de cada 4 pacientes. La prednisona fue el fármaco más utilizado con una dosis media de 40 mg.

Subgrupo de pacientes con edad> 65

- Este subgrupo de pacientes tiene factores de riesgo diferentes en comparación con la población general, con la misma edad: la mayoría de ellos no tenían comorbilidades, y el diagnóstico fue realizado en forma ambulatoria.

- Los episodios fueron más frecuentes en la CU de localización rectal. La EII fue diagnosticada a una edad más tardía, y tenía una corta evolución (media 4 años) en el momento de la aparición de la infección.

- En la EC, la implicación del colon fue más frecuente en los episodios de ICD

- El diagnóstico de EII y la infección con CD se produjo al mismo tiempo en el 20% de los episodios y la mitad de ellos requirieron hospitalización.

- IBP fueron más frecuentes en los episodios CDI como un factor de riesgo.

- El tratamiento inmunosupresor y biológico en el momento de los episodios fueron frecuentes: 40% y 30% respectivamente. 1 de cada 3 episodios tenían dos combinados.

- Hay una tendencia a la escalada terapéutica, hospitalización, cirugía, complicaciones y la mortalidad en los pacientes de este subgrupo con la infección.

- Presentan tasas de recurrencias son altas (30%), lo que justificaría que estos pacientes fueron tratados con un antibiótico que tuviera una menor tasa de recurrencia.

- La población de estudio es pequeña y se necesitan estudios más amplios para confirmar estos resultados.

Factores de riesgo independientes para la ICD en los pacientes con EII en el estudio multivariante

- Los factores de riesgo independientes para tener una infección por CD son los siguientes: CU, infección que coincide con el diagnóstico de la EII, antibióticos tres meses previos a la aparición del episodio y comorbilidades.

- Los IBP fueron un factor de riesgo en el análisis univariante pero no consiguieron significación estadística en el análisis multivariante.

Variables en la ecuación

		B	E.T.	Wald	gl	Sig.	Exp(B)	I.C. 95% para EXP(B)	
								Inferior	Superior
Paso 4[a]	InfeyDx(1)	,981	,502	3,818	1	,051	2,666	,997	7,129
	Antibprevi(1)	1,430	,545	6,884	1	,009	4,178	1,436	12,158
	tipoenf(1)	1,155	,305	14,300	1	,000	3,173	1,744	5,772
	Comorbilidad(1)	,934	,357	6,857	1	,009	2,544	1,265	5,118
	Constante	-3,471	,796	19,023	1	,000	,031		

a. Variable(s) introducida(s) en el paso 4: InfeyDx.

Puntos clave en el estudio de los episodios recurrentes.

- En nuestro estudio, las recurrencias fueron frecuentes (18%) y más de la mitad aparecieron 8 semanas del primer episodio. Después de la primera recurrencia (13%) había una mayor probabilidad de una segunda (3%) y una tercera (2%).

- Encontramos que la segunda recurrencia fue más precoz (< 8 semanas) que la primera y la tercera (> 8 semanas).

- Los episodios recurrentes fueron más frecuentes en la CU izquierda y extensa. Encontramos la infección en la EC con afectación colónica, apareciendo un 50% en aquella con localización perianal.

- En nuestro medio encontramos que la CU recurre más de 1 vez pero no encontramos más de una recurrencia en la EC.

- Un 13% de los episodios ocurrieron en pacientes mayores de 65 años

- El 88% de los episodios recurrentes ocurrieron en pacientes ambulatorios, con adquisición en la comunidad de un 100%.

- 1 de cada 4 pacientes fue tratados con antibióticos 3 meses antes (100% con metronidazol). Más del 70% de los pacientes fue tratado con IBP 3 meses antes de la aparición del episodio.
- Comorbilidad ≥1, 20%. El tipo de severidad más frecuente fue la de leve-moderada.

- En el momento de la ICD un 75% de los episodios estaban siendo tratados con tratamiento combinado: inmunosupresores y/o biológicos y/o aféresis.

- En el momento de la infección los pacientes con CU estaban con menos tratamientos inmunosupresor y biológico con respecto a la EC. Después de una recurrencia la CU tuvo más escalada terapéutica y hospitalización que la EC.

- No encontramos colectomías tras un año de la recurrencia.

- Tratamos los episodios recurrentes de la misma manera que los primeros episodios. Más de un 90% fueron tratados con metronidazol y menos de un 10% fueron tratados con vancomicina.

- El tratamiento con corticoides se llevó a cabo en más de un 80% de los episodios recurrentes: El fármaco más utilizado fue la prednisona con una dosis media de 40 mg al día

- No encontramos complicaciones o muertes a causa de la infección por Clostridium difficile.

Factores de riesgo independientes para la recurrencia de la ICD.

En el análisis multivariante encontramos como factores de riesgo para tener una ICD recurrente: tener una CU, estar en tratamiento con inmunosupresores y fármacos biológicos.

Variables en la ecuación

		B	Error estándar	Wald	gl	Sig.	Exp(B)	95% C.I. para EXP(B)	
								Inferior	Superior
Paso 1[a]	TtoIS(1)	1,457	,480	9,208	1	,002	4,294	1,675	11,006
	Constante	,754	,303	6,182	1	,013	2,125		
Paso 2[b]	tipoenf(1)	1,484	,613	5,859	1	,015	4,413	1,326	14,680
	TtoIS(1)	1,680	,503	11,146	1	,001	5,368	2,001	14,396
	Constante	,234	,360	,424	1	,515	1,264		
Paso 3[c]	tipoenf(1)	2,286	,812	7,925	1	,005	9,831	2,002	48,267
	TtoIS(1)	1,753	,522	11,282	1	,001	5,769	2,075	16,041
	ttobiolo(1)	2,692	,962	7,827	1	,005	14,761	2,239	97,305
	Constante	-2,373	1,036	5,243	1	,022	,093		

a. Variables especificadas en el paso 1: TtoIS.

b. Variables especificadas en el paso 2: tipoenf.

c. Variables especificadas en el paso 3: ttobiolo.

10.7 Conclusiones

- No hemos encontrado un incremento en el número de casos de infección por Clostridium difficile en nuestro Hospital desde junio de 2007 hasta junio de 2014. La frecuencia de esta infección en los pacientes con enfermedad inflamatoria intestinal no está caracterizada en España. A consecuencia de su baja prevalencia son necesarios estudios prospectivos y multicentricos para evaluar la incidencia y prevalencia de esta infección.

- Una comunicación fluida y directa con el Servicio de Microbiología es crucial para conocer los resultados positivos de forma más precoz y así poder tomar las decisiones terapéuticas más adecuadas tan pronto como sea posible.

- No deberíamos pedir de forma rutinaria muestras de heces en todas las recidivas desde el inicio del cuadro clínico. Sólo se deberían solicitar cuando el paciente presente factores de riesgo para la infección y en todos los pacientes hospitalizados. Además las deberemos pedir cuando el paciente no responda a la optimización o intensificación del tratamiento basal y al tratamiento corticoideo.

- No es necesario confirmar la resolución microbiológica con muestras de heces de control tras el tratamiento si el paciente ha mejorado.

- La infección por Clostridium difficile fue frecuente durante los primeros 3-7 años después del diagnóstico de la enfermedad inflamatoria intestinal (50% y más del 70% respectivamente). Una cuarta parte de los episodios ocurrió durante el primer año tras el diagnóstico de la enfermedad. La EII, por si sola, es un factor de riesgo para la infección por Clostridium difficile y es posible que al diagnóstico de la EII, la inflamación no esté bien controlada aún y esto constituya un factor predisponente para la ICD.

- El comportamiento de la colitis ulcerosa (CU) y la enfermedad de Crohn (EC) con o sin infección por Clostridium fue diferente: la ICD hace que el comportamiento de la CU sea similar al comportamiento de la EC sin Clostridium difficile. La CU con la infección tiene mayor posibilidad de recidiva de forma más temprana que la CU sin infección, donde la evolución sólo depende de su evolución natural. Sin embargo, en la EC las recidivas fueron menos frecuentes en comparación con la EC sin infección, en donde las recidivas dependían de la evolución natural de la enfermedad sin un desencadenante externo de la recidiva.

- La infección por Clostridium difficile es una enfermedad prevenible y tratable. El control de los factores de riesgo de la infección (profilaxis) y una temprana detección de la misma son fundamentales para la prevención y el tratamiento precoz, respectivamente.

- Debemos tener un alto grado de sospecha:

    - En pacientes con enfermedad inflamatoria intestinal activa colónica. (CU y EC con localización colónica). Hemos encontrado que la ICD es más frecuente en CU con localización rectal. La CU es un factor de riesgo independiente para la ICD.

- En el diagnóstico de la enfermedad inflamatoria intestinal: en ese momento hay más riesgo para la infección por Clostridium difficile. Hemos encontrado que se trata de un factor de riesgo independiente para la infección. Estos resultados podrían apoyar el papel que el Clostridium difficile puede jugar en la aparición de la EII.

- Después de cualquier episodio de ICD: los pacientes con EII tienen una mayor probabilidad de recurrencia, especialmente después de las primeras ocho semanas tras finalizar el tratamiento. La mayoría de los episodios ocurrieron en personas jóvenes y fueron adquiridos en la comunidad. El diagnóstico fue hecho como paciente ambulatorio. Además, los pacientes con EII tienen un perfil de riesgo diferente si los comparamos con la población general, y la EII por si solo pudiera jugar algún papel en la ICD.

- La mayoría de los episodios de ICD fueron leves-moderados. Necesitamos establecer marcadores pronósticos para determinar el riesgo de desarrollar una infección grave mediante la realización de estudios prospectivos y multicentricos. ESCMID guía clínica para la población general, considera que la EII por si sola es un marcador pronóstico de una infección por Clostridium difficile severa.

- En nuestro estudio, en pacientes españoles con EII, los factores de riesgos independientes en el análisis multivariante para la infección por Clostridium difficile fueron: CU, coincidencia al diagnóstico de la EII, antibióticos tres meses antes del episodio y la presencia de comorbilidades. Los IBP tres meses antes del episodio, fueron un factor de riesgo en el análisis univariante.

- La infección por CD se trata de una enfermedad prevenible y tratable. Dos de estos factores de riesgo son prevenibles (antibióticos e IBP), otro se puede sospechar precozmente (al diagnóstico de la EII) y la comorbilidad no es modificable pero si mejorable. Además, la identificación de los factores de riesgos modificables, evitar un sobreuso de los IBP y tener un tratamiento antibiótico escalonado juega un papel esencial en la prevención de los episodios por ICD en los pacientes con EII.

- El porcentaje de hospitalización fue bajo, pero los pacientes con EII necesitaron de manera más significativa más hospitalización. La mayoría de los episodios tuvieron una severidad moderada. Así nosotros debemos mejorar la comunicación con el Servicio de Urgencias para hospitalizar sólo aquellos pacientes que lo necesiten. El uso de IBP y antibióticos de amplio espectro debe ser cuidadoso en los pacientes hospitalizados.

- No encontramos una peor evolución, pero los episodios de Clostridium difficile tienen una tendencia a una mayor escalada terapéutica 6 meses después de los mismos. No encontramos episodios con cirugía un año después. Más aún, no tenemos más complicaciones o muertes asociadas a la ICD por si misma.

- En nuestro estudio no encontramos que los tratamientos inmunosupresores y biológicos fueran un factor de riesgo para un único episodio de infección por Clostridium difficile. Además no encontramos una mayor proporción de tratamiento combinado. Nosotros no encontramos diferencias en la evolución de los pacientes con infección por Clostridium en

tratamiento inmunosupresor y biológico en los episodios de infección por Clostridium difficile.

- Hemos encontrado los siguientes factores de riesgo independiente en el análisis multivariante para la recurrencia de la ICD en los pacientes con EII: colitis ulcerosa, tratamiento inmunosupresor y biológico.

- La calprotectina fecal pudiera jugar un papel en la clasificación de estos pacientes en grupos de riesgo y su seguimiento. Necesitamos más esturdíos para determinar el valor real de este marcador biológico en el manejo de la infección por Clostridium difficile. Sin embargo los niveles de albumina y creatinina no resultaron ser útiles para elaborar estos grupos de riesgo ya que en la mayoría de los casos aparecían en cifras dentro de la normalidad.

- En nuestro hospital la mayoría de los pacientes eran de mediana edad, sin embargo el subgrupo de paciente de más de 65 años parece tener un comportamiento diferente con peor evolución, pronóstico y mayor número de recidivas. Así deberíamos empezar el tratamiento con vancomicina oral en estos pacientes.

- Tanto el metronidazol como la vancomicina resultan igual de eficaces, sin embargo la vancomicina resulta más útil para tratar episodios recurrentes y evitar recurrencias.

- Los resultados de nuestro estudio pueden ser útiles para definir grupos de riesgo en la ICD, además nuevas intervenciones se requieren para prevenir los episodios (tratamiento apropiado y prevenir/evitar factores de riesgo).

- En nuestra opinión sería necesario la creación de un grupo de trabajo multidisciplinario para desarrollar guías clínicas para el manejo de la ICD en los pacientes con EII: diagnóstico, tratamiento y estrategias de prevención en Europa.

10.8 Recomendaciones basadas en nuestras conclusiones

- Debemos sospechar una sobreinfección por CD en el momento del diagnóstico inicial de una EII, para iniciar el tratamiento específico tan pronto como sea posible. Es obligatorio, al diagnóstico de una EII, hacer un diagnóstico diferencial con otras causas de diarrea infecciosa, como la infección por Clostridium difficile.

- Debemos tener un alto grado de sospecha en pacientes con EII activa de localización colónica al momento del diagnóstico y durante su evolución.

- Habría que optimizar el tratamiento de base añadiendo tratamiento local y aumentar de la dosis de mesalazina oral.

- A menudo, optimizando el tratamiento se va a controlar la recaída y no sería necesario solicitar muestras de heces para investigar la presencia del CD.

- Eliminar los factores de riesgo y controlar a los individuos de alto va a permitir la profilaxis o la detección temprana de CD.

- El primer paso del manejo de la CU con ICD sería optimizar el tratamiento oral y local con mesalazina y tratamiento antibiótico. Sería esencial explicar al paciente la importancia de su adhesión al tratamiento tópico.

- Uno de los principales objetivos del tratamiento de la EII es evitar el tratamiento con corticoides. Nosotros no pudimos, no tratar con corticoides: episodios leves-moderados

(episodios sin severidad clínica o en los parámetros de laboratorio). Además, sería beneficioso para el paciente comenzar con tratamiento sólo en los episodios leves sin riesgos de complicaciones o recurrencias. Necesitamos estudios controlados prospectivos de tratamiento de la infección por Clostridium en los pacientes con EII. En los pacientes hospitalizados parece razonable comenzar con corticoides intravenosos y metronidazol o vancomicina mientras esperamos por el resultado de los test de Clostridium. Aunque los pacientes de Consulta externa en mejores condiciones la decisión puede esperar hasta la confirmación de la infección por las muestras de heces.

- Necesitamos elaborar protocolos específicos para tratar la ICD en los paciente s con EII de forma específica. Así, probablemente después de una recurrencia, episodios severos y pacientes con alto riesgo de recurrencia deberíamos utilizar vancomicina como primera opción terapéutica: En nuestra área la CU recurre más que la EC. Así en el tratamiento de la CU debemos utilizar vancomicina, al menos después de la primera recurrencia. Más aun, ¿deberíamos tratar con vancomicina todas las ICD en pacientes con CU desde los primeros episodios o solo en el subgrupo de pacientes hospitalizados, en riesgo de recurrencia o en episodios severos? Necesitamos estudios prospectivos y multicentricos en pacientes con EII para definir los grupos de riesgo con peor evolución y evaluar las mejoras estrategias para ellos basados en la edad del paciente, marcadores biológicos de severidad y comorbilidades.

- Necesitamos estudios prospectivos y controlados para evaluar las mejores opciones de tratamiento en cada subgrupo de nuestros pacientes con EII basados en el riesgo de recurrencia, el número de episodios y la severidad.

10.9 Discusión de nuestros resultados

Nos gustaría destacar en este resumen nuestras conclusiones sobre el manejo terapéutico de la infección por Clostridium difficile. Quizá es el aspecto en el que tendríamos que introducir cambios en nuestro manejo y establecer protocolos de actuación nuevos-

10.9.1 Tratamiento de la infección por Clostridium difficile

En este resumen nos ha parecido más importante destacar los aspectos relativos al tratamiento. Necesitamos estudios prospectivos, controlados para valorar la mejor aproximación terapéutica en los pacientes con EII e infección por CD. Es más, necesitamos estudios prospectivos y multicentricos en pacientes con EII (debido a su baja prevalencia) para definir grupos de riesgo basados en la severidad clínica y parámetros de laboratorio y de acuerdo a esto, definir las mejores opciones terapéuticas.

En nuestra opinión hay dos aspectos esenciales en el manejo de estos pacientes: profilaxis y manejo precoz de la infección. El primer paso en el manejo de la CU con infección por Clostridium difficile sería optimizar el tratamiento oral y rectal con mesalazina, además del tratamiento antibiótico. En nuestro estudio, solo uno de cada tres pacientes fue tratado con mesalazina rectal en el momento de la infección. Tras optimizar el tratamiento de base añadiendo tratamiento tópico rectal incrementamos la dosis de mesalazina oral. Un paciente puede tener una recidiva si no sigue el tratamiento (en el caso de una colitis ulcerosa distal, no usando tratamiento local). A menudo, la optimización del tratamiento es suficiente para controlar la recidiva y no es necesario investigar la presencia de toxina de CD en las heces del paciente. Así, seria crucial que los médicos explicaran a los pacientes la importancia de la adhesión al tratamiento local.

En nuestro centro se suele utilizar, de forma rutinaria el mismo tratamiento antibiótico (metronidazol oral) en la mayoría de los episodios ya sea el primero o sucesivos: leve-moderado o severo (aunque la mayoría de ellos fueron leve-moderado). Sin embargo, tuvimos un 18% de recurrencia. Más aún, nosotros combinamos el tratamiento antibiótico con corticoides en más de un 70% de los pacientes. >En 1 de cada 3 pacientes la prednisona fue el medicamento de elección, con una dosis media de 40 mg.

Uno de los principales objetivos del tratamiento de la EII es evitar el uso concomitante de corticoides. Así en el paciente con EII infectado por Clostridium sería interesante comenzar únicamente con el tratamiento antibiótico en los episodios leves sin riesgo de complicación o de recurrencia. Sin embargo, nos encontramos con el problema de definir la severidad del episodio y hasta ahora no disponemos de guías específicas para tal fin. Así el tratamiento antibiótico solo para la infección por Clostridium difficile en los pacientes con EII con una recidiva grave no puede ser recomendado.

En los pacientes hospitalizados con un episodio moderado-severo parece razonable comenzar el tratamiento con corticoides intravenosos y metronidazol o vancomicina mientras esperamos los resultados de las muestras de heces. Sin embargo, la decisión terapéutica puedes ser demorada en espera de resultados en los pacientes que acuden por Consultas Externas con episodios leves-moderados, pudiendo comenzar con la optimización del tratamiento basal del paciente. Así podríamos no tratar con corticoides: episodio leve-moderado o episodios sin severidad clínica o en los parámetros de laboratorio. Por todas estas razones, las estrategias para el majo de la infección por Clostridium difficile en los pacientes con EII, sería acorde con la edad de la paciente marcadores biológicos de severidad y la presencia de comorbilidades. Pero ¿Cuál es el mejor tratamiento antibiótico? El tratamiento con metronidazol y vancomicina efectivo en más de un 88% de los casos. Así, la mayoría de los pacientes responden inicialmente

al tratamiento antibiótico pero un 10-40% pueden tener una recurrencia posterior asociada a la presencia de Clostridium en las heces. La ICD es difícil de tratar y la recurrencia tras un primer episodio es alta, por lo que estaría justificado un tratamiento más específico en caso seleccionado.

En nuestros opinión el punto clave es no solo el tratamiento de los episodios, metronidazol y vancomicina son igual de eficaces a la hora de tratar la infección pero a la hora de prevenir la recurrencia la vancomicina ha mostrado ser mejor. Además probablemente después de la primera o siguiente recurrencia en los episodios severos y pacientes con alto riesgo de recurrencia deberíamos usar vancomicina como primera opción terapéutica.

Sin embargo, estas recomendaciones se aplican a la población general pero no de forma específica a la población con EII. Necesitamos estudios prospectivos, multicentricos y controlados para evaluar la mejor opción terapéutica para cada subgrupo de nuestros pacientes con EII basados en el número, severidad y riesgo de recurrencia de los episodios.

Los episodios más frecuentes en nuestro estudio fueron con severidad leve-moderada y se usó como primera línea de tratamiento el metronidazol oral (más de un 90%) ya sea en los primeros episodios como en las recurrencias. La vancomicina y los probióticos fueron usados en un porcentaje más bajo.

La guía de la Sociedad Europea de Microbiología Clínica y Enfermedades Infecciosas (ESCMID), en la sección de recomendación de tratamiento antibiótico oral para el primer episodio en una infección no severa recomienda: metronidazol, 500 mg 3 veces al día durante 10 días (grado A) como primera opción y vancomicina 125 mg 4 veces al día durante 10 días (grado B). Además en la última frase, mencionan una opción diferente de tratamiento: “la no administración de antibióticos o su retirada y observar la respuesta clínica del paciente durante

48 horas" (grado C) (ver apéndices: tabla 4). Así les tratamos a nuestros pacientes según los criterios de una guía clínica diseñada para población general. Sin embargo, la última frase en esta sección es muy interesante porque si pudiéramos tener el resultado de las muestras en 24 h, sería posible antes que nada optimizar el tratamiento de la enfermedad inflamatoria intestinal mientras esperamos el resultado de las muestras.

Este punto es también importante en nuestro caso ya que nuestros pacientes no usaban tratamiento tópico en el momento del episodio y por otro lado podríamos evitar el uso de corticoides orales. Nosotros usamos una combinación de tratamientos antibióticos y corticoides en más de un 70% de los pacientes. La prednisona fue el corticoide más utilizado con una dosis media de 40 mg al día. Así sería necesario comenzar tan pronto como sea posible con el tratamiento específico para la infección y evitar el uso de corticoides en los casos leves. No tenemos estudios prospectivos específicos en los pacientes con EII donde este procedimiento haya sido estudiado.

Tenemos un ratio bajo de episodios severos, pero su tratamiento fue el mismo en que el caso de los episodios leve-moderado. La recomendación de las guías es utilizar vancomicina a dosis de 125 mg cuatro veces al día durante 10 días o vancomicina 500 mg cuatro veces al día durante 10 días. Como tratamiento de primera elección ya que tiene un ratio de cura más alto que el metronidazol en esta situación (grado A) (ver apéndices: tabla 5).

Nuestro porcentaje de recurrencias fue alto pero concordante con la literatura. Usamos el mismo tratamiento en todos los casos: metronidazol oral en un 90% de los episodios. La guía recomienda como tratamiento para la primera recurrencia: vancomicina 125 mg cuatro veces al día durante 10 días (grado B) o fidaxomicina 200 mg dos veces al día durante 10 días (grado B). Y como tratamiento de las múltiples recurrencias (más de una): vancomicina a dosis de 125 mg cuatro veces al día durante 10 días seguida de un régimen pulsado (125-500 mg/día cada 2-

3 días) durante al menos tres semanas (grado B) o vancomicina dosis de 125 mg cuatro veces al día durante 10 días seguida de un régimen de descenso gradual de la dosis a 125 mg/día. Este subgrupo de paciente necesita ser tratado para evitar más recurrencias (ver apéndices: tablas 6 y 7).

Identificar los pacientes con EII con un mayor riesgo para la infección por CD y la prevención o diagnóstico temprano es el primer paso. En nuestra área son factores de riesgo independientes para la infección: la colitis ulcerosa, el momento del diagnóstico de la EII, ingesta de antibióticos tres meses antes de los episodios y la presencia de comorbilidades. Además, aparecen como factores de riesgo en el análisis univariante: la toma de inhibidores de la bomba de protones tres meses antes de la aparición del episodio. En los episodios recurrentes fueron factores de riesgo independiente la colitis ulcerosa y el tratamiento inmunosupresor y biológico. Basándonos en nuestro estudio podríamos cambiar nuestra estrategia terapéutica (ver capítulo 6: nuestras propuestas para modificar el manejo de la infección por Clostridium difficile en los pacientes de nuestra área).

Necesitamos marcadores pronósticos específicos para los pacientes con EII, a fin de mejorar el tratamiento de la infección por Clostridium difficile en este tipo de pacientes. Si estudiásemos con detalle las recomendaciones de la Guía de la Sociedad Europea de Microbiología, los marcadores pronósticos utilizados fueron: edad superior a 65 años, leucocitosis mayor de 15.000 l/c, albumina inferior a 3 gr, aparición de insuficiencia renal y la presencia de comorbilidades. En esta sección, se consideró a la enfermedad inflamatoria intestinal por sí misma un factor de riesgo para desarrollar una infección severa por Clostridium difficile (ver apéndices: tabla 2). Así, si seguimos las guías generales, la EII per se es un marcador pronóstico para el desarrollo de una infección severa. Por lo tanto, necesitamos guías específicas para el tratamiento de la ICD en los pacientes con EII.

Hay pocos datos y escasos estudios controlados sobre la eficacia de otros tratamientos para la ICD en los pacientes con EII como los probióticos o el trasplante fecal, ambos utilizados más frecuentemente en la población general; el trasplante fecal en episodios severos o recurrentes. Sin embargo estos datos no han sido investigados en los pacientes con EII con infección por CD. En nuestra opinión el trasplante fecal parece una opción viable para el tratamiento tanto de la infección por Clostridium difficile como de la propia enfermedad inflamatoria intestinal per se, sin embargo esto deberá ser confirmado mediante la elaboración de estudios controlados.

10.9.2 Subgrupo de pacientes con más de 65 años

Este subgrupo supone menos de un 10% de los episodios de ICD pero su comportamiento parece ser interesante. Ellos no tuvieron comorbilidades y el diagnóstico fue hecho como paciente ambulatorio a diferencia de la población general con esta edad.

La EII tuvo el mismo comportamiento que en los pacientes más jóvenes: más frecuente en CU y EC con localización colónica. El diagnóstico de la EII fue hecho con más de 40 años de edad y la infección ocurrió durante los primeros cinco años de evolución de la enfermedad. Encontramos que un 20% de los episodios aparece en el momento del diagnóstico de la EII y que la mitad de ellos requirió hospitalización.

El uso de IBP fue frecuente en los pacientes con la infección por Clostridium difficile, no así el uso de antibióticos. El porcentaje de recurrencias fue alto (30%) lo cual podría justificar que este subgrupo fuese tratado con antibióticos con menor porcentaje de recurrencias. En el momento de los episodios había un porcentaje más elevado de tratamiento inmunosupresor y biológico (40% y 30% respectivamente) y uno de cada 3 pacientes tuvo tratamiento combinado.

Ellos tuvieron tendencia a la escalada terapéutica. Un bajo porcentaje de los episodios requirió hospitalización, cirugía y tuvo complicaciones y mortalidad en los pacientes con la ICD. Este subgrupo es pequeño y se necesitarían estudios más grandes para confirmar estos resultados.

Aportaciones originales de nuestro estudio

Nuestro estudio tuvo las siguientes limitaciones: fue un estudio retrospectivo desarrollado en un único centro con una pequeña muestra. Revisamos las historias clínicas en papel siendo muy dificultosa la extracción de ciertos datos acerca de la severidad de la enfermedad. Además los parámetros de laboratorio no fueron solicitados en todas las recidivas por sus médicos responsables.

A pesar de estas limitaciones, nuestros hallazgos son similares a los obtenidos en estudios previos y subrayan las variables más importantes a considerar durante la valoración de los riesgos y opciones de tratamiento en un primer episodio o en episodios recurrentes de infección por Clostridium difficile.

Nuestro estudio es el primero en evaluar, más que factores de riesgo en pacientes con EII en España. También valoramos recurrencia y evolución. Nuestros hallazgos podrían tener implicaciones para el tratamiento y control de la infección por CD en nuestra área y podría ayudar a establecer un perfil de riesgo para la infección por CD en los pacientes con EII.

Este estudio es un primer paso en la investigación de la infección por CD en los pacientes con EII. Sería importante la creación de grupos de trabajo multidisciplinarios para la elaboración de estudios multicentricos a nivel europeo y crear protocolos para el diagnóstico, tratamiento y prevención de la infección en este tipo de pacientes.