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Helicobacter pylori eradication therapy is not associated with the onset of inflammatory bowel disease. A case-control study

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To my Parents and to Reza

Bibliography description

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Abstract

Background & Aims: A negative association between *Helicobacter pylori (H. pylori)* and inflammatory bowel disease (IBD) has been previously reported. There were also case reports suggesting a new onset of IBD 6-12 months after *H. pylori* eradication therapy. In a case-control study we investigate whether previous *H. pylori* eradication therapy is associated with the risk of developing IBD.

Methods: IBD outpatients with both Crohn´s disease (CD) and ulcerative colitis (UC) were enrolled. Age- and sex-matched blood donors served as controls in a 1:2 fashion. Information on demographics, medical history, previous *H. pylori* infection and eradication therapy was recorded. Serum samples for *H. pylori* serology testing (anti-*H. pylori*-IgG and anti-CagA-IgG) were obtained. Controls that received *H. pylori* eradication therapy during the 12 months previous to enrollment were excluded.

Results: Overall, 127 IBD patients (CD N= 90; UC N= 37) and 254 controls were enrolled. The prevalence of *H. pylori* infection (positive *H. pylori* serology and/or previous eradication) in IBD patients and controls was 11% and 23%, respectively (OR 0.4, 95% CI 0.21-0.74, p<0.003). Four patients (3%) developed IBD (3 MC and 1 CU) after receiving successful *H. pylori* eradication (latency 6-12 months). The rate of previous *H. pylori* eradication therapy in patents who successively developed IBD was lower but not statistically different from that observed in the control group (OR 0.43, 95% CI 0.14-1.29, p=0.16).

Conclusions: In our study previous *H. pylori* eradication therapy was not associated with the onset of IBD. Whether in a subgroup of patients *H. pylori* eradication therapy may trigger a latent IBD cannot be excluded.

Keywords: Crohn´s disease (CD), ulcerative colitis (UC), *Helicobacter pylori*

(*H. pylori*), inflammatory bowel disease (IBD);

Abstract in German

Hintergrund: Studien haben eine niedrige *H. pylori*-Seroprävalenz bei chronisch-entzündlichen Darmerkrankungen (CED) Patienten gezeigt, was auf eine protektive Rolle dieser Infektion bei der Entwicklung von CED hindeuten könnte. Drei klinische Fällberichte berichten über eine Erstmanifestation einer CED nach *H.-pylori*-Eradikationstherapie.

Ziel: Die Assoziation zwischen *H. pylori* Eradikationstherapie und dem Risiko der Entwicklung einer CED zu untersuchen.

Material und Methoden: In einer Fall-Kontroll-Studie wurden konsekutiv ambulante CED-Patienten mit Morbus Crohn (MC) und Colitis Ulcerosa (CU) eingeschlossen. Alters- und geschlechtsangepasste Blutspender dienten als Kontrollen. *H. pylori* Serologie (anti-*H. pylori*-IgG und anti-CagA-IgG) wurden bei allen Studienteilnehmer entnommen. Informationen über Demographie, Anzahl der Geschwister und frühere *H.-pylori*-Infektion und Eradikationstherapie wurden bei CED Patienten und Kontrollen erfasst.

Ergebnisse: 127 CED-Patienten und 254 Kontrollen wurde eingeschlossen. Die *H.-pylori* Seroprävalenz lag bei 11% bei den CED-Patienten und bei 23% bei den Kontrollen (OR 0.4, 95% CI 0.21-0.74, $p<0.003$). Die Rate einer anamestischen *H. pylori* Eradikationstherapie war ähnlich zwischen beiden Gruppen (10% bei CED-Patienten und 7% Kontrollen, OR 1,49, 95% CI 0.70-3.15). Vier Patienten entwickelten eine CED 6-12 Monate nach einer erfolgreichen *H. pylori* Eradikationstherapie (3 MC und 1 CU).

Zusammenfassung: In dieser Studie konnte keine Assoziation einer *H. pylori*-Eradikationstherapie mit dem Auftreten einer CED gezeigt werden. Weitere Studien sollten klären, ob in einer Subgruppe die *H. pylori*-Eradikationstherapie eine subklinische CED auslösen kann.

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List of Abbreviations

^{13}C urea breath test	^{13}C-UBT
5-aminosalicylic acid	5-ASA
Bismuth quadruple therapy	BQT
Crohn´s Disease	CD
Crohn's Disease Activity Index	CDAI
Cytotoxin associated antigen	CagA
Enzyme-linked immunosorbent assay	ELISA
Esophagogastroduodenoscopy	EGD
Harvey-Bradshaw Severity index	HBSI
Helicobacter pylori	*H. pylori*
Horseradish peroxidase	HRP
Inflammatory bowel disease	IBD
Lymphoma of the mucosa - associated lymphoid tissue	MALT
Magnetic Resonance Imaging	MRI
Muramyl-dipeptide	MDP
Non-steroidal anti-inflammatory drugs	NSAIDs
Nucleotide-binding oligomerization domain containing 2	NOD2
Protonpump inhibitors	PPI
Rapid urease test	RUT

Regulatory T cells	Treg
Type 1 T helper	Th1
Type 2 T helper	Th2
Type 4 secretion system	T4SS
Ulcerative colitis	UC
Vacuolating cytotoxin	VacA

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1. INTRODUCTION

1.1 Inflammatory Bowel Disease

1.1.1 Definition

Inflammatory bowel disease (IBD) is a chronic, idiopathic, relapsing inflammatory auto-immune disorder of the gastrointestinal tract [1]. Ulcerative colitis (UC) and Crohn's disease (CD) are the two major forms of IBD.

Crohn's disease is a relapsing, transmural inflammatory disease of the gastrointestinal mucosa that can affect the entire gastrointestinal tract from the mouth to the anus. Typical presentations include the discontinuous involvement of various portions of the gastrointestinal tract and the development of complications including strictures, abscesses, or fistulas [2]. Ulcerative colitis is a relapsing non-transmural inflammatory disease that is restricted to the colon. It affects the rectum and to a variable extent the colon in a continuous fashion [3].

1.1.2 Epidemiology

Worldwide the incidence and prevalence of IBD significantly increased in the 21th century [4]. It is currently estimated that about 1 –1.3 million people suffer from IBD in the United States [5] and 3.7 million in Europe [6].

In Europe the estimated prevalence of CD is 213 per 100.000 adults and of UC 294 per 100.000 [6]. The incidence rate are from 3.6 to 6.3 cases per 100.000 person/years for CD and 8 to 11.4 cases per 100.000 person/years for UC respectively [6].

Although there are few epidemiologic data from developing countries, the incidence and prevalence of IBD is increasing with time and in different regions of the world indicating its emergence as a global disease (Figure 1) [4].

The peak age of incidence of CD is in the third decade of life, with a decreasing incidence rate with age [7]. The incidence rate in UC is quite stable between the

third and seventh decades [7]. Regarding sexual distribution, there is a slight preponderance of UC in males, while CD is more frequent in women [8] .

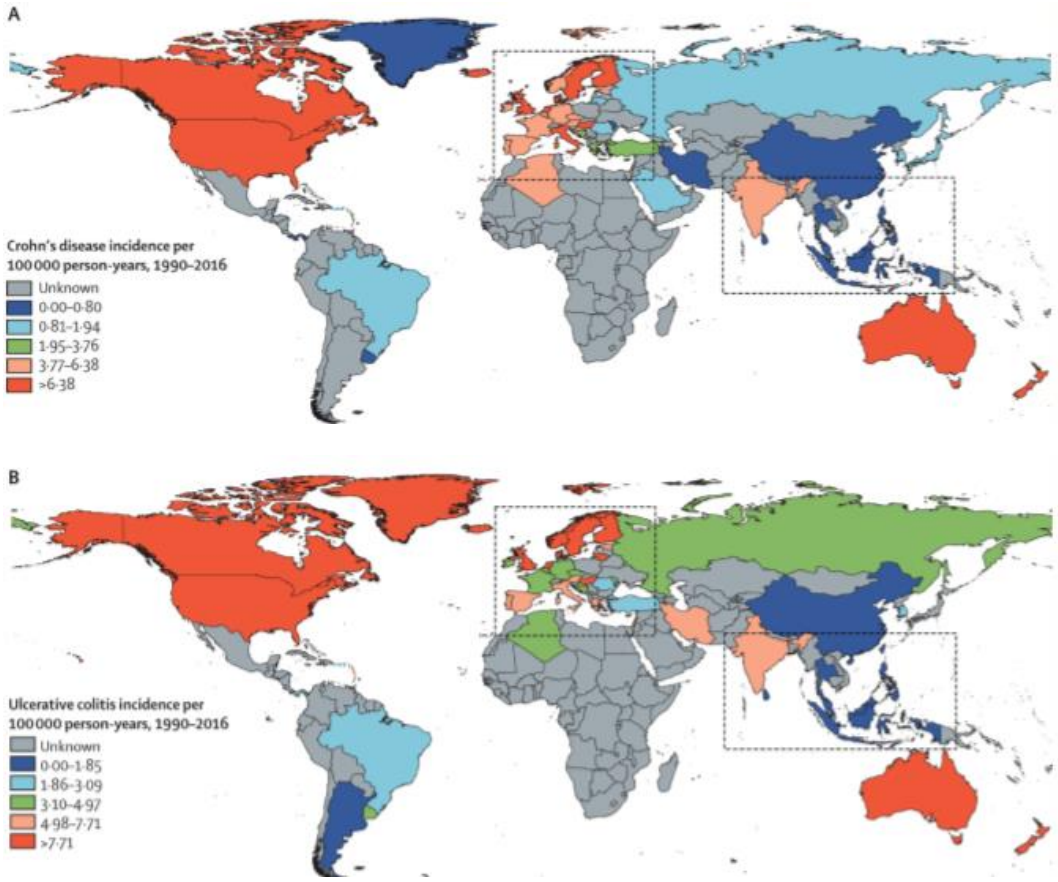

Figure 1. A: Crohn´s disease worldwide incidence; B: Ulcerative colitis worldwide incidence [4].

1.1.3 Pathogenesis

IBD is a complex, multifactorial disorder characterized by a chronic relapsing intestinal inflammation. Although the etiology remains largely unknown, recent research has suggested that personal genetic susceptibility, environment, intestinal microbiota and immune system are all involved in the pathogenesis of IBD.

1.1.3.1 Genetics

The IBD genetic research began with the discovery of NOD2 (nucleotide-binding oligomerization domain containing 2), that codes for a protein originally described as an intracellular receptor recognizing the muramyl-dipeptide (MDP), a conserved motif present in peptidoglycan from both gram-positive and -negative bacteria [9].

Patients who carry heterogeneous NOD2 genes have a two-four times higher risk of developing CD, whereas those with homogeneous alleles have 20–40 fold increased risk [10]. Moreover, the NOD2 gene has been associated with ileal involvement, stricturing disease, earlier age of onset and a positive family history of CD [10].

1.1.3.2 Environment

Environmental factors increasing the risk of IBD include smoking, diet, drugs and social stress. Contrary to its protective effect on UC, smoking is associated with a twofold increased risk for CD [11]. Moreover, smoking is also associated with early onset of CD, more frequent need of immunosuppression, more surgical interventions and higher rates of post-operative disease recurrence [12].

High dose, prolonged duration and frequent use of non-steroidal anti-inflammatory drugs (NSAIDs) had been associated with an increased risk of IBD [13]. Stress also plays a role in the pathogenesis of IBD [14]. Subjects with lower levels of stress had a reduced risk of disease onset. Anxiety or depression are associated with relapse, hospitalization, surgery, reduced efficacy of immunomodulators and impaired quality of life [15]. Appendectomy done for perforating appendix is associated with higher risk for developing CD [16].

1.1.3.3 Microbiome

IBD patients have reduced diversity in gut microbiota when compared with healthy individuals [17]. Many studies have examined the gut flora in

both inflamed and non-inflamed segments and reported a significantly reduced biodiversity in fecal microbiome in IBD patients [18].

Firmicutes and *Bacteroidetes* phyla are predominatly found in healthy individuals and contribute to the production of epithelial metabolic substrates. In CD, the microbiota is characterized by a lack of *Firmicutes* and *Bacteroidetes* and an over-representation of *Enterobacteria* [19]. A reduction in *Clostridium spp*. and an increase in *Escherichia coli* have been reported in UC [19].

1.1.3.4 Immune Response

The intestinal immune system is the largest and most complex component of the immune system in human beings. Environmental factors may trigger the onset of IBD by altering the intestinal mucosal barrier and the healthy balance of the gut microbiota, thus abnormally stimulating the gut immune responses [20].

As the intestine comprises the major single epithelial interface in the body and the greatest number and diversity of resident microbes, the intestinal immune system must discriminate between invasive organisms and harmless antigens (Figure 2, panel a) [20]. The disturbance of this balance and the dysfunctions of innate and adaptive immune pathways contribute to the aberrant intestinal inflammatory response in patients with IBD. During the initial phase of the inflammation, a foreign unknown antigen activates the intestinal innate immune cells (Figure 2, panel b). A maintained inflammatory reaction promotes the activation of the adaptive immune response.

Abnormally activated effector CD4+ T helper (Th) cells synthetize and release different inflammatory mediators that generate a circle of inflammation resulting in chronic tissue injury and epithelial damage (Figure 2, panel c) [21].

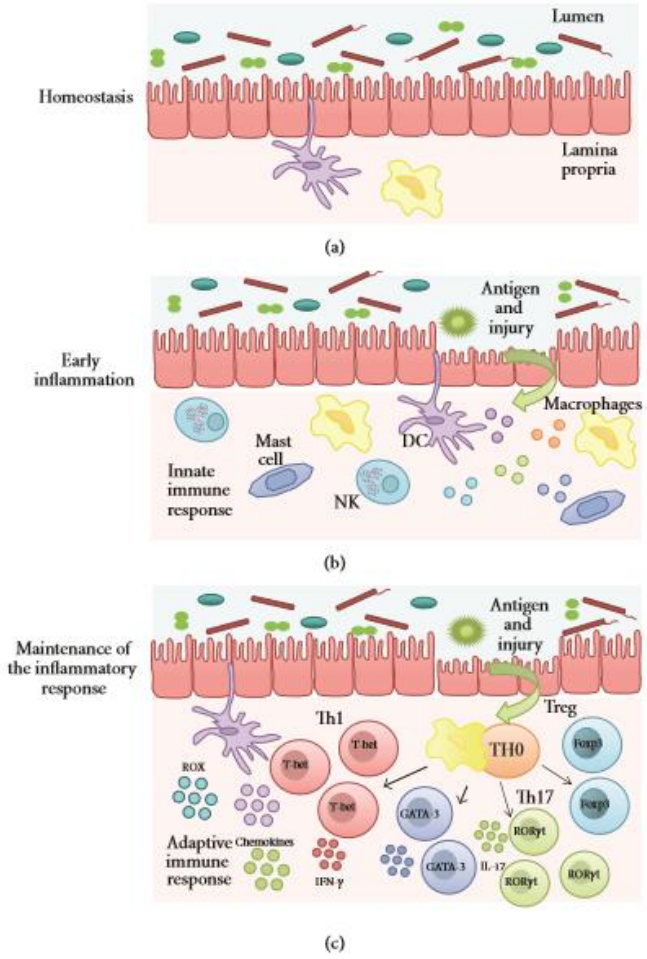

Figure 2. Immune response in IBD [20].

1.1.4 Clinical Features

UC patients typically present with bloody diarrhea mixed with pus and/ or mucus, accompanied by abdominal cramps during the bowel movements [22]. The location of abdominal pain depends on the extent of colonic involvement. Associated symptoms of urgency or tenesmus may be present [22]. The clinical presentation of CD is depending on disease location and includes abdominal pain, weight loss, clinical signs of bowel obstruction and diarrhea [23]. Systemic symptoms of fatigue, anorexia or fever are common. At the diagnosis the disease is located in the colon in 25%, ileum in 25% and ileocolon in 50%. Proximal

gastrointestinal involvement occurs in 0.5-16% of patients [24]. Perianal disease is present in 4–10% of patients at the time of diagnosis as anal tags, deep anal fissures or fistulae [25]. Extraintestinal manifestations are usually related to the intestinal disease activity and may precede or develop the intestinal symptoms [26]. In Table 1 are listed the most common extraintestinal manifestations.

Table 1. Common extra-intestinal manifestations in IBD [26].

Extraintestinal manifestations
Musculoskeletal manifestations
– peripheral arthritis (type I:pauciarticular arthritis; type II: polyarthritis)
– axial arthropathies (ankylosing spondylitis/Bekhterev's disease with sacroiliitis/enthesitis)
Dermatological manifestations
– pyoderma gangrenosum
– erythema nodosum
Ocular manifestations
– anterior/posterior uveitis
– episcleritis/scleritis
Hepatobiliary manifestations
– primary sclerosing cholangitis (PSC)
– autoimmune hepatitis (AIH)
– overlap syndrome/autoimmune cholangitis

1.1.5 Clinical Scores- Crohn´s disease

Several IBD scoring systems have been developed to classify and characterize IBD patients, with the goal of helping to better define the disease status and effectiveness of therapy. The Crohn's Disease Activity Index (CDAI) is a research tool used to quantify the symptoms of patients with CD in order to record disease activity. The index consists of eight factors, each added up after adjustment with a weighting factor [27]. The Harvey-Bradshaw Severity index (HBSI) was devised as a simplified version of the CDAI for data collection purpose [28]. It consists of only 5 clinical parameters:

1. general well-being (0 = very well, 1 = slightly below average, 2 = poor, 3 = very poor, 4 = terrible)
2. abdominal pain (0 = none, 1 = mild, 2 = moderate, 3 = severe)
3. number of liquid stools per day
4. abdominal mass (0 = none, 1 = dubious, 2 = definite, 3 = tender)
5. complications (1 point for each): arthralgia, arthritis, uveitis, erythema nodosum, pyoderma gangrenosum, aphthous ulcers, anal fissures, fistulae or abscesses, other fistulae, fever during the previous week

A score of less than 5 points is generally considered to represent clinical remission.

1.1.6 Clinical Scores- ulcerative colitis

The Mayo score is the most common used activity index in UC [29]. The score comprises four categories rated from 0–3 that are summed to give a total score that ranges from 0–12. A clinical version of the score without endoscopic evaluation is the partial Mayo score that analyzed only 3 parameters:

1. Stool frequency (0= normal, 1= 1-2 stools/day more than normal, 2= 3-4 stools/day more than normal, 3= >4 stools/day more than normal)
2. Rectal bleeding (0= None, 1= visible blood with stool less than half the time, 2= visible blood with stool half of the time or more, 3= passing blood alone)
3. Physician rating of disease activity (0= normal, 1= mild, 2= moderate, 3= severe)

A partial Mayo score of less than 2 points is considered to represent clinical remission.

1.1.7 Pathology

In UC, the inflammatory infiltrate is limited to the intestinal mucosa and is composed of lymphocytes, plasma cells and neutrophils, causing typically cryptitis, defined as the presence of neutrophils within crypt epithelium, and crypt abscesses, defined as the presence of neutrophils within crypt lumina [30]. Based on three features, namely an increase of lymphocytes and plasma cells in the lamina propria, presence of crypt branching and cryptitis UC can be distinguished from other inflammation [31].

A focal discontinuous chronic inflammation, focal crypt irregularity with discontinuous crypt distortion and granulomas are the accepted microscopic features for a diagnosis of CD. It is characterized either by crypt distortion, crypt branching or crypt shortening [32]. Granuloma in CD is defined as a collection of epithelioid histiocytes, such as monocyte/macrophage cells. Only the granulomas in the lamina propria not related to crypt injury are considered a feature of CD. In fact, non-caseating granulomas can be observed in infectious colitis from *Mycobacterium sp., Chlamydia sp., Yersinia pseudotuberculosis, Treponema sp.* [33].

1.1.8 Diagnosis

The diagnosis of IBD is established by a combination of medical history, clinical evaluation, laboratory, exclusion of infectious pathogens and typical endoscopic, histologic and radiologic findings [23].

A full history investigates travel anamnesis, medication, especially intake of antibiotics and NSAIDs, smoking, family history, previous appendectomy and recent episodes of infectious gastroenteritis. In details, it should consider daily stool frequency and consistency, urgency, rectal bleeding, abdominal pain, malaise, fever, weight loss and symptoms of extra-intestinal manifestations of IBD.

Physical examination includes general well-being, measurement of weight and calculation of body mass index, pulse rate, blood pressure, temperature,

abdominal tenderness or distension, palpable abdominal masses and perineal examination [34]. Laboratory investigations comprise full blood count, urea and electrolytes, liver function tests and erythrocyte sedimentation rate or C reactive protein [35] , ferritin, transferrin saturation, vitamin B12 and folate [36]. Fecal calprotectin is accurate in detecting colonic inflammation and can help to distinguish functional diarrhea [37]. Microbiological testing for *Clostridium difficile* toxin, in addition to standard organisms, is essential [38].

Colonoscopy with multiple biopsies allows classification of disease based on endoscopic extent, severity of mucosal inflammation and histological features [39]. Upper gastrointestinal endoscopy is considered in coexisting dyspepsia to exclude upper gastrointestinal manifestation of CD. Sonography in expert hands has a high sensitivity for detecting disease and Doppler techniques are useful in the assessing the degree of disease activity [40]. Moreover, it is the first line diagnostic detecting abscess or other CD complication [41]. Magnetic resonance imaging (MRI) has very high sensitivity in diagnosing of small bowel involvement of CD, extraluminal complications (including abscess formation) and demonstrates internal fistulisation with good accuracy [42]. Pelvic MRI has a particular place in the evaluation of perianal disease [43].

1.1.9 Treatment

Treatment strategies are complex, consisting of pharmacological treatment and surgery depending on disease location, severity and patients' treatment history [44].

The traditional first-line therapy with "conventional" treatments consists of aminosalicylates, corticosteroids and immunomodulators (e.g. azathioprine, 6-mercaptopurine) [45]. The use of biologic agents to induce remission in patients with moderate to severe disease or inadequate response to conventional therapy entered the IBD-guidelines [23, 46].

The indications for surgical treatment in CD include complex inflammatory changes along the entire small and large bowel, with the formation of abscesses,

fistulae to the skin, to the bladder and to other segments of the gastrointestinal tract. Aim of any surgical intervention is the removal of stenosis or inflammatory foci, including drainage of abscesses and excision of fistulae, with maximal preservation of the intestinal function [47]. In UC aside from absolute surgical indications, such as bowel perforation, severe bleeding, toxic megacolon with or without perforation and colon carcinoma, the most common reasons for surgical treatment are an intractable disease course or the development of dysplasia [46].

1.2 Helicobacter pylori

1.2.1 Epidemiology

At least half the world's population is infected by *H. pylori* making it the most widespread infection in the world. A recent systematic review showed that in 2015, approximately 4.4 billion individuals worldwide were *H. pylori* positive [48]. Prevalence is highest in Africa (79.1%), Latin America and the Caribbean (63.4%), and Asia (54.7%). In contrast, *H. pylori* prevalence is lowest in Northern America (37.1%) and Oceania (24.4%) [49]. In Saxony-Anhalt, (Germany), the estimated prevalence is 28.9% [50]. The differences in *H. pylori* prevalence reflect directly the level of urbanization, sanitation, access to clean water and socioeconomic status. The infection is usually acquired in early childhood. Moreover, the socioeconomic status during childhood, such as the level of education and the economical income of the parents, as well as the status of sanitary conditions has a significant impact on the incidence of *H. pylori* [51].

1.2.2 Principal virulence factors

Electron microscopy studies on gastric biopsy specimens have shown that *H. pylori* has a specific tropism for the intercellular junctions and expresses, among others, the outer membrane protein adhesin, which binds to lipids and carbohydrates on the epithelial cell membrane allowing *H. pylori* to adhere to the epithelial cells (Figure 3) [52].

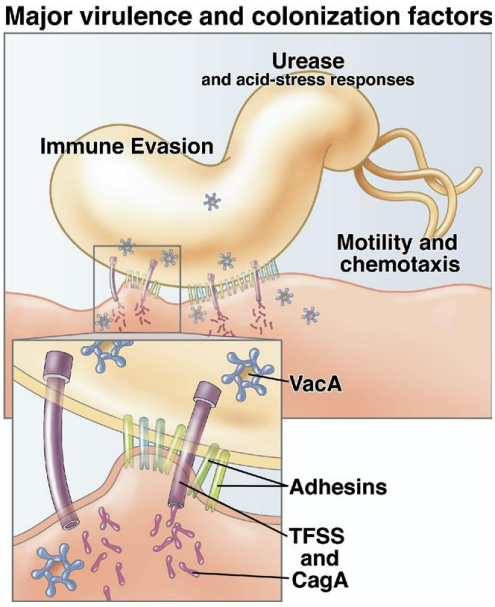

Figure 3. *H. pylori* major virulence and colonization factors [53]**.**

1.2.2.1 Cytotoxin associated antigen (CagA)

The cytotoxin associated antigen (CagA) is the most extensively studied *H. pylori* virulence factor. There are two types of *H. pylori*: the CagA-producing (CagA positive) strains and CagA-non producing (CagA negative) strains [54]. In Western countries, subjects infected with CagA positive strains of *H. pylori* are at a higher risk of peptic ulcer or gastric cancer than those infected with CagA negative strains [54]. The gene encoding for CagA is part of the so-called Cag pathogenicity island, which in addition to the CagA also encodes the proteins of the type 4 secretion system (T4SS).

Following the attachment of *H. pylori* to gastric epithelial cells, the CagA protein is delivered into gastric epithelial cells through the bacterium type 4 secretion system (T4SS) [55]. Then the CagA is phosphorylated in the host cell and influences different signaling pathways. This leads, for example, to a change in the cytoskeleton and to an enlargement of the cells. The cell-cell contacts are also disturbed and the cell polarity is abolished. The morphological changes of the epithelial cells observed thereafter are described as "hummingbird phenomenon" [53]. CagA can also interact with intracellular

proteins and deregulate distinct signaling pathways through both tyrosine phosphorylation-dependent or independent mechanisms. The pathobiological effects of CagA determinate also the promotion of inflammation and dysregulation of proliferation and apoptosis [53].

1.2.2.2 Vacuolating cytotoxin (VacA)

All *H. pylori* strains contain a vacuolating cytotoxin (VacA) gene, but not all strains produce a functional VacA toxin. This is due to polymorphisms within the VacA gene, particularly at the amino-terminus (s region), at the middle (m region) and at intermediate region (i region) of the gene. The s2 polymorphism yields an inactive toxin [56]. VacA binds to a receptor-like protein tyrosine phosphatases (RPTPα and RPTPβ) and other glycosylated transmembrane proteins on the host cell surface [57]. VacA is then endocytosed and forms anion-selective channels in vacuole membranes. These channels allow accumulation of chloride anions and weak bases, resulting in osmotic swelling [58]. Furthermore, VacA exerts many other functions, which is why it is called multifunctional toxin. For example, VacA inserts into mitochondrial membranes, causing mitochondrial dysfunction and subsequent apoptotic death of the cell [59]. VacA can also disrupt the barrier function of epithelial cells, allowing leakage of crucial nutrients such as iron, nickel, and aminoacids, improving *H. pylori* growth. Finally, it has also immunomodulatory capabilities interfering with the B cell function and inhibiting the T cell proliferation [60] .

1.2.3 H. pylori – associated diseases

Usually the infection with *H. pylori* occurs in childhood. A brief phase of diffuse acute inflammation of the gastric mucosa is followed by a chronic infection. All infected individuals develop type B chronic active gastritis, but most of them will never develop symptoms. The further clinical course varies depending on bacterial and host factors [61].

H. pylori is responsible for 90 - 100% of duodenal ulcers and 60 - 100% of gastric ulcers [62]. IARC classified *H. pylori* as a class 1 carcinogen [63].
H. pylori accounts for 63% of gastric cancers and for 5.5% of global cancer cases [64]. MALT lymphoma (lymphoma of the mucosa - associated lymphoid tissue) occurs in less than 1% of *H. pylori* infected [65].

1.2.4 Diagnosis

Diagnostic tests for *H. pylori* infection include invasive and non-invasive methods. The invasive methods are based on biopsies taken during esophagogastroduodenoscopy (EGD) and include culture, histology, rapid urease test (RUT) and PCR. Non-invasive methods are the ^{13}C urea breath test (^{13}C-UBT), the stool antigen test and serology [66, 67].
Both ^{13}C-UBT and stool antigen test are good non-invasive approaches for the diagnosis of *H. pylori* infection, with high sensitivity and specificity [68].
Serological tests can be used only after validation [67]. This can be done by testing the serum of patients known to *be H. pylori* positive by invasive methods (histology, culture, PCR). In fact, these tests may perform differently in different geographic locations according to the antigenic composition of the circulating strains [69].
In clinical practice when there is an indication for endoscopy and there is no contraindication for biopsy, the rapid urease test (RUT) is recommended as a first-line diagnostic test. Both sensitivity and specificity of RUT are of 90%, is in the range of 90–95% [70].
In the assessment of *H. pylori* gastritis, a minimum standard biopsy setting is two biopsies from the antrum (greater and lesser curvature 3 cm proximal to the pyloric region) and two biopsies from the middle of the body [71]. Additional biopsy from the incisura is considered for a higher accuracy in detecting precancerous lesions and assessing the risk of developing gastric cancer [72].
^{13}C-UBT is the best option for confirmation of *H. pylori* eradication and it should be performed at least 4 weeks after completion of therapy [67]. The stool antigen

test is also a good alternative. Because serology is able to detect past infection with *H. pylori* it should not be used as a method to monitor effectiveness of eradication.

1.2.5 Treatment

The triple treatment including proton pump inhibitors (PPI)-clarithromycin and amoxicillin or metronidazole over 7 days allows the cure of only a maximum of 70% of the patients [73]. This low eradication rate is the consequence of several factors: reduced compliance of the patients, high gastric acidity, high bacterial load, type of strains and increased resistance to clarithromycin. Actually the clarithromycin resistance rate has reached a prevalence >20% in most countries of Central, Western and Southern Europe [74]. Therefore, the current European guidelines recommend in areas of high (>15%) clarithromycin resistance the use of the bismuth quadruple therapy (BQT) for 10 days consisting of a PPI, a bismuth, metronidazole and tetracycline [67]. In areas of low clarithromycin resistance, a PPI-clarithromycin-amoxicillin triple therapy extended to 14 days is recommended as first-line empirical treatment [67]. After failure of the triple therapy, a BQT or a fluoroquinolone-containing triple or quadruple therapy are recommended as a second-line treatment. After failure of a second-line treatment, culture with susceptibility testing or molecular determination of genotype resistance is recommended in order to guide treatment [67].

1.2.6 Helicobacter species and IBD

Depending on the site of the gastrointestinal tract colonized, *Helicobacter* species are divided into entero-hepatic *Helicobacters* (EHH) and gastric *Helicobacters* [75]. Different studies have investigated the association between IBD and *Helicobacter* species. In case-control studies, both UC [76] and CD [77] were associated with the presence of EHH species DNA in intestinal biopsies.

Various studies have consistently shown a low *H. pylori* seroprevalence in patients with IBD, suggesting a protective role of this infection in the development of the disease [8]. Moreover, epidemiological data demonstrate that IBD is more prevalent in areas with lower rates of *H. pylori* infection. In attempting to explain this negative association between *H. pylori* infection and IBD, different hypotheses have been suggested but the issue still remains a controversial subject. A possible protective role of *H. pylori* infection in the development of autoimmune diseases such as asthma or IBD was suggested. The mechanism underlying this presumed protective role of *H. pylori* infection in autoimmune disorders is the result of a local mucosal inflammatory response, which might elicit a systemic release of cytokines inducing immune tolerance and limiting inflammatory responses [78].

A study found a lower prevalence of *H. pylori* in IBD patients treated with sulfasalazine [79]. However, a following study did not support this concept as treatment with sulfasalazine or any other medical therapy (i.e. 5-aminosalicylic acid (5-ASA), steroids, thiopurines, antibiotics) had no influence on *H. pylori* prevalence [80].

Recently, three clinical cases reported an IBD onset after *H. pylori* eradication therapy [81] [82] [83]. According to the most widely accepted hypothesis, environmental factors may trigger IBD onset in individuals with genetic susceptibility by altering the intestinal mucosal barrier and the healthy balance of the gut microbiota, resulting in an aberrant immune response of the gut [84].

H. pylori dominates the microbiota community of the gastric mucosa and influence duodenal and oral communities [85]. Changes in microbial diversity and loss of protective microbial species have been demonstrated in patients with IBD [86]. In particular, antibiotic exposure has been shown to alter gut microbiota. Recently a population-based case-control study in Olmsted County (Minnesota) found a statistically significant association between antibiotic use and new-onset IBD [87]. The hypothesis that *H. pylori* eradication therapy (i.e. the intake of two antibiotics and a PPI) could alter the microbial diversity and thus abnormally stimulate the gut immune response in genetically susceptible subjects is

fascinating. On the other hand, the loss of *H. pylori* induced immune response may play a role in increasing the susceptibility to develop IBD as well. These aspects may lead to anxiety in patients and even induce the physicians to refrain from prescribing appropriate *H. pylori* eradication therapy.

1.3 Aim of the study

The etiology of IBD is poorly understood [88]. Various studies consistently have shown a low *H. pylori* seroprevalence in patients with IBD, suggesting a protective role of this infection in the development of IBD [89]. Recently, three clinical cases reported an IBD onset after *H. pylori* eradication therapy [81–83]. Accordingly, the hypothesis that a profound change of the intestinal microbiota induced by eradication therapy (i.e. a combination of two antibiotics and a PPI) or the loss of *H. pylori* induced immune response may be the trigger for IBD appears attractive.

Our aim was to study whether previous *H. pylori* eradication therapy may be associated with an increased risk of developing IBD. The prevalence of previous *H. pylori* eradication - the putative risk factor - was studied in cases (IBD patients) and controls.

2. MATERIAL AND METHODS

2.1 Study population

2.1.1 Patient group

Patient with IBD were prospectively enrolled during the period from December 2016 and Mai 2017 at the outpatient Department of Gastroenterology, Hepatology and Infectious Diseases at the Otto-von-Guericke University (Magdeburg, Germany).

2.1.2 Control group

Sex- and age-matched (± 5 years) controls were selected from a cohort of 516 consecutive healthy blood donors that were enrolled between May and June 2016 in a prospective epidemiological study on seroprevalence of *H. pylori* infection in Saxony-Anhalt [50]. Demographics, clinical information and serum samples of blood donors were available.

2.2 Study design

The primary aim of the present study was to evaluate whether previous *H. pylori* eradication therapy may represent a risk factor for developing IBD. For answering this question we chose a case-control study design in a 1:2 fashion. The prevalence of previous *H. pylori* eradication therapy - the putative risk factor - was studied in IBD patients (cases) and controls.

2.2.1 Inclusion end exclusion criteria

2.2.1.1 Patient group

Inclusion criteria were the presence of a confirmed IBD and a written informed consent.

Patients with missing written informed consent were excluded from the analysis. To simplify the recruitment, only IBD patients receiving a biological therapy (i.e. Infliximab, Vedolizumab and Ustekinumab) were enrolled. In our clinic, biological therapy for IBD patients is scheduled on Fridays (ca. 25 patients per day). In addition, all participants were subject with a minimum age of 18 years.

2.2.1.2 Control group

All healthy blood donors were ≥ 18 years old and had already signed a written informed consent.

Subjects that received *H. pylori* eradication therapy less than 12 months before enrolment were excluded from matching, as cases of new-onset IBD reported in the literature were diagnosed 6-12 months after eradication therapy.

2.2.2 Study protocol

2.2.2.1 Patient group

IBD Patients were interviewed using structured questionnaire that comprised information on demographics, number of siblings (no siblings, <3 siblings and >3 siblings), medical history and previous *H. pylori* eradication therapy. Study participants with previous *H. pylori* eradication therapy provided records of the eradication including the regimen used for eradication therapy and the test performed to confirm successful eradication. Moreover, IBD onset was also recorded. IBD activity was

clinically evaluated by the Mayo Clinic score and the Harvey-Bradshaw Severity Index for patients with UC and CD, respectively [30].

Enrolled patients received a physical examination and medical history was collected. After insertion of a peripheral venous line for biological therapy, blood samples for routine laboratory tests and *H. pylori* serology were obtained.

Study participants who were seropositive for *H. pylori* infection and/or self-reported previous *H. pylori* infection and successful eradication therapy were considered *H. pylori*-positive. We choose this definition to include the largest number of patients with current or past *H. pylori* infection.

The questionnaire and the informed consent are shown in annexes 1 and 2.

2.2.2.2 Control group

Sex- and age-matched (± 5 years) controls were selected from a cohort of 516 consecutive healthy blood donors that were enrolled between May and June 2016 in a prospective epidemiological study on the seroprevalence of *H. pylori* infection in Saxony-Anhalt [50].

Controls were interviewed using structured questionnaire that comprised information on demographics, number of siblings, medical history and previous *H. pylori* eradication therapy. Study participants with previous *H. pylori* eradication therapy provided records of the eradication including the regimen used for eradication therapy and the test performed to confirm successful eradication.

In addition to routine diagnostics, one serum sample was taken for *H. pylori* laboratory testing. Study participants who were seropositive for *H. pylori* infection and/or self-reported previous *H. pylori* infection and successful eradication therapy were considered *H. pylori* positive. We chose this definition to include the largest number of subjects with current or past *H. pylori* infection.

2.3 *H. pylori* serology

Serum blood samples from case and control were analyzed by an enzyme-linked immunosorbent assay (ELISA).

The ELISA is an antibody-based method based on an enzymatic color reaction. The serological detection of *H. pylori* infection was carried out with the *H. pylori* IgG ELISA kit (Biohit, Helsinki, Finland) and the CagA-IgG ELISA kit (Genesis Diagnostics, Cambridgeshire, UK). Both methods were performed according to the manufacturer's recommendation.

By way of example, we will shortly describe the protocol of *H. pylori* IgG ELISA kit.

The ELISA is based on the technique of enzyme immunoassay with partially purified *H. pylori* antigen. This was fixed on a microtiter plate and determined by adding an HRP (horseradish peroxidase) labeled antibody (Biohit Oyj, *H. pylori* IgG, Instructions for use, Helsinki, Finland).

For the *H. pylori* IgG ELISA (Biohit, Helsinki, Finland), the following reagents were used per sample: 6x350 µl wash buffer concentrate; 100 µl dilution buffer; 100 µl calibrator; 100 µl negative control; 100 µl positive control; 100 µl conjugate solution; 100 µl substrate solution and 100 µl stop solution.

For the preparation, the microtiter plates and all reagents were brought to room temperature (23 °C) and the wash buffer concentrate diluted 1 in 10 with distilled water. The patient serum separated after coagulation was diluted 1 to 200 with the dilution buffer and then mixed. 100 µl each of the dilution buffers, the calibrator, the negative control, the positive control and the diluted samples were individually pipetted into the microtiter plates as a duplicate determination. At the polystyrene surface of this plate was purified *H. pylori* antigen, which could bind the *H. pylori* antibody from the sample. After covering the plate with cover film, the incubation was performed for 30 min at 37 °C.

In the following step, the strips were cleaned with 3x350 µl of diluted wash solution. Subsequently, 100 µl of the mixed dilution (1 to 100) of the conjugate solution in the wells with an 8-channel pipette, the plate covered again and

incubated at 37 °C for 30 min. During this time, the HRP-labeled monoclonal non-human IgG was able to bind the *H. pylori* antibodies from the sample. The following procedure included the renewed cleaning with dilute washing solution (3x350µl). To the substrate solution, 100 µl of this reagent (TMB substrate) were added to all emptied wells using an 8-channel pipette and the batch was incubated for 30 min at room temperature. During this process, the plate was protected from light. The substrate could now be oxidized by the enzyme, resulting in a blue color change. To complete the enzyme reaction, 100 µl of stop solution were pipetted into the wells and the absorbance at 450 nm was then measured. The confirmation of the correct test procedure was indicated by the absorbance value of the calibrator. This value should be >1000 U/ml. The mean absorption value could be determined by the following formula:

$$\frac{X(Asample) - X(ABlank)}{X(ACalibrator) - X(ABlank)} \times 100 = \text{Probe} U/ml\,(\text{enzyme-units})$$

Serum samples that had a value greater than 30 U/ml and whose samples turned yellow were anti-*H. pylori* IgG positive classified. For the CagA-IgG ELISA kit (Genesis Diagnostics, Cambridgeshire, UK) patients >6.25 U/ml were considered positive for anti-CagA IgG.

Materials: patient blood from serum tube; *H. pylori* IgG ELISA (Biohit, Helsinki, Finland): wash buffer concentrate (120 ml 10x concentrated phosphate buffer solution with Tween 20 and 0.1% ProClin as preservative), dilution buffer (100 ml phosphate buffer with blocking protein, Tween 20, 0.1% ProClin300 as preservative and red dye) Calibrator (contains 1.5 ml of human serum-based H. pylori IgG calibrator with 0.1% Proclin300 as preservative), negative control (contains 1.5 ml of human serum-based *H. pylori* IgG negative control with 0.1% Proclin300 as a preservative), positive control (contains 1.5 ml of human serum-based *H. pylori* IgG positive control with 0.1% Proclin300 as preservative), conjugate solution (0.2 ml of horseradish peroxidase, (HRP) -coupled monoclonal non-human IgG in stabilization buffer at 0.02 % methylisothiazolone, 0.02%

bromonitrodioxane and 0.002% active isothiazolone as preservative), substrate solution (15 ml tetramethylbenzidine i.p. n aqueous solution), 100 µl stop solution (15 ml 0.1 mol / l sulfuric acid); CagA IgG ELISA kit (Genesis Diagnostics, Cambridgeshire, United Kingdom).

2.4 Ethical statement

The study protocol was conducted according to the declaration of Helsinki [90] and approved by the ethics Committee of the Otto-von-Guericke University Hospital of Magdeburg (protocol number 80/11). A detailed description of the study project including patient information was submitted to the local ethics committee under the name:

"Adaptive *Helicobacter pylori*- assoziierte bakterielle und Wirtssuszeptibilitätsfaktoren in der Magenkarzinogenese“

The approval from the Ethics committee can be consulted in Annex 3.

2.5 Case control matching

We assigned to each IBD patient two age (± 5 years) and sex matched controls (healthy blood donors). Controls were first selected after gender and thereafter according to age in order to give to each patient the closest or the second closest matched control (± 5 years).
For the subgroup analysis each patient with CD or UC was matched with two controls, again first after gender (male/female) and thereafter according to age (± 5 years).

2.6 Statistical analysis

Sample size calculation with a power of 80% and a desired significance level of 5% was performed with an online calculator (http://osse.bii.a-star.edu.sg/calculation1.php). Given that the eradication rate in the cohort of 516 blood donors was of 5.4% and the number of cases eradicated before IBD onset unknown, the sample size needed for cases and controls in a 1:2 fashion was 105 and 210, respectively.

Distribution of the demographic characteristics and related factors were compared by the Mann–Whitney U test for continuous data and by Fisher´s exact test for categorical variables, which were performed using an online calculator (https://www.graphpad.com/quickcalcs/ttest1.cfm). A statistical p value of 0.05 (two sided) was considered significant for all comparisons.

For the analysis of the association between *H. pylori* infection and IBD onset, estimated odds ratios (OR) with corresponding 95% confidence intervals (CIs) were calculated (https://www.easycalculation.com/statistics/odds-ratio.php).

3. RESULTS

3.1 Summary

Information on *H. pylori* serological status and epidemiological data was analyzed in IBD patients and matched controls.

Furthermore, subgroup analyses of the predefined categories were performed to compare UC *vs*. CD as well as CD *vs.* controls and UC *vs.* controls.

All patients and controls were German, living in Magdeburg or its neighborhood, in the constituent state of Saxony-Anhalt.

3.2 Demographics, medical therapy and clinical scores in IBD patients

Overall, 127 IBD patients (71 M: 56 F, median age 42, mean age 40.12±12.01 years, range 18-72 years) were prospectively enrolled between December 2016 and May 2017. Ninety of them had CD (34 M: 56 F, mean age 41.5±12.02 years, range 18-72 years), 37 had UC (22 M: 15 F, mean age 42±11.98 years, range 18-67 years) and none had unclassified IBD (IBDU).

Fifteen IBD patients (10 CD and 5 UC) had a mild disease, defined by a Mayo Clinic score of 2 to 5 points and a Harvey-Bradshaw Severity Index of 6 or 7 points. All other IBD patients were in clinical remission (Mayo Clinic score of less than 2 points and a Harvey-Bradshaw Severity Index lower than 5).

To simplify the recruitment, only IBD patients receiving a biological therapy (i.e. Infliximab, Vedolizumab and Ustekinumab) were enrolled. In details, 36 (40%) patients with CD and 15 (16%) with UC were treated with Infliximab with or without 5-ASA. A therapy with Vedolizumab with or without 5-ASA was performed in 10 (11%) CD and 8 (8%) UC patients. Five (5%) CD patients and 1 (1%) UC patient (off-label use) received a therapy with Ustekinumab with or without 5-ASA. A combined immunosuppressive therapy was defined by a combination of thiopurines (i.e Azathioprine or 6-mercaptopurine) with biological

therapy with or without 5-ASA. Overall, 23 (25%) CD patients and 8 (8%) UC patients received a combined therapy with thiopurines and Infliximab with or without 5-ASA, whereas 13 (14%) CD patients and 4 (4%) UC patients received a combined therapy with thiopurines and Vedolizumab with or without 5-ASA. Finally, three CD patients (3%) and 1 UC patient (1%) were treated with thiopurines and Ustekinumab with or without 5-ASA.

A detailed description of demographics, medical therapy and clinical scores in patients with IBD at study enrollment is reported in Table 2.

Table 2. Demographics, medical therapy and clinical scores in patients with inflammatory bowel diseases (IBD) at study enrollment.

CD: Crohn's disease, UC: ulcerative colitis; •Mayo Clinic score: remission defined as a score < 2 points, mild disease defined as a score of 2 to 5 points; * HBSI: Harvey-Bradshaw Severity Index, remission defined as score ≤ 5, mild disease defined as a score of 6 to 7 points; ˜ Thiopurines: Azathioprine or 6-mercaptopurine.

	CD (%)	**UC (%)**
Patients (N)	90	37
Male/female	34/56	22/15
Median age (years), (range)	41.5±12 (18-72)	40±11.9 (18-67)
Mayo Clinic score•: mild colitis/remission	-	5/32
HBSI*: mild colitis/remission	10/80	-
Biological therapy ±5-ASA		
Infliximab ± 5-ASA	36 (40)	15 (16)
Vedolizumab ± 5-ASA	10 (11)	8 (8)
Ustekinumab ± 5-ASA	5 (5)	1 (1)
Thiopurines˜ + biological therapy ± 5-ASA		
Thiopurines +Infliximab ± 5-ASA	23 (25)	8 (8)
Thiopurines +Vedolizumab ± 5-ASA	13 (14)	4 (4)
Thiopurines+Ustekinumab ± 5-ASA	3 (3)	1 (1)

3.3 IBD patients *vs.* controls

3.3.1 Epidemiological data

We performed an analysis considering IBD patient (N=127) and two sex- and age (±5 years) matched healthy blood donors (N=254). In this investigation, 71 (56%) out 127 IBD patients were male and 56 (44%) female, median age 42±12.0 years. No significant difference was found regarding gender and age of IBD patients and blood donors. The percentage of study participants that had no siblings was similar between IBD patients and matched blood donors (20% vs. 20%, respectively, OR 0.89, 95% CI 0.55-1.63, p=0.95). Similarly, the percentage of study participants that had less than three siblings did not differ between IBD patients and controls (64% vs. 65%, respectively, OR 0.9, 95% CI 0.61-1.15, p=0.96). Finally, also the percentage of IBD patients and controls reporting to have more than three siblings was similar (16% vs. 15%, OR 0.88% CI 0.55-1.63, p=0.95). A detailed description of the demographics data is reported in Table 3.

Table 3. Comparison of demographics data, in patients with inflammatory bowel diseases (IBD) and controls.

*:p< 0.05, °: Mann–Whitney U test for continuous data and by Fisher´s exact test for categorical variables; ç: odds ratios (OR) with corresponding 95% confidence intervals.

	IBD (127)	**Controls (254)**	***p*°**	**OR (CI 95%)ç**
Male/Female	71/56	132/122	NS	
Median age ± Standard Deviation (years)	42±12.0	41±12.4	NS	
Siblings (%)				
No Siblings	24 (20)	50 (20)	0.89	0.95 (0.55-1.63)
< 3	82 (64)	166 (65)	0.9	0.96 (0.61-1.5)
≥ 3	21 (16)	38 (15)	0.88	1.06 (0.58- 1.91)

3.3.2 H. pylori serological status

Overall, 11% (14 out of 127) of patients with IBD had a positive serology for *H. pylori*. Increased titers of anti- *H. pylori* -IgG, anti-CagA-IgG or both were observed respectively in 10 (7%), 6 (5%) and 2 (1, 5%) IBD patients.

Twenty-one out of 127 IBD patients (16%) and 73/254 controls (29%) were *H. pylori*-positive (positive *H. pylori* serology and/or previous eradication therapy) (OR 0.4, 95% CI 0.28-0.84, p=0.01). Four out of 127 patients developed an IBD (3 CD and 1 UC) after *H. pylori* eradication therapy. An inverse association between *H. pylori* infection and IBD (OR 0.4, 95% CI 0.21-0.74, p<0.003) was observed. Detailed information on the statistical analysis is reported in Table 4.

Table 4. Comparison of *H. pylori* serology and history of *H. pylori* eradication therapy in patients with inflammatory bowel diseases (IBD) and controls.

[a]: anti- *H. pylori* IgG titer >30 EIU; [b]: anti-CagA titer >6.25 U/mL; [c]: defined as positive anti- *H. pylori* IgG or positive anti-CagA or positive medical history for *H. pylori* eradication; *:p< 0.05, °:Mann–Whitney U test for continuous data and by Fisher´s exact test for categorical variables; [c]:odds ratios (OR) with corresponding 95% confidence intervals.

	IBD (127)	**Controls (254)**	***p*°**	**OR (CI 95%)[c]**
H. pylori positive (%) [a]	10 (7)	63 (24)	0.0001*	0.25 (0.12-0.52)
H. pylori CagA positive [b] (%)	6 (5)	31 (12)	0.02*	0.35 (0.14-0.87)
H. pylori positive IgG and CagA (%)	2 (1.5)	23 (9)	0.003*	0.16 (0.03-0.69)
H. pylori positive IgG and/or CagA (%)	14 (11)	60 (23)	0.003*	0.4 (0.21-0.74)
H. pylori positive IgG and/or CagA and/or previous *H. pylori* Eradication (%)[c]	21 (16)	73 (29)	0.01*	0.4 (0.28-0.84)
Previous *H. pylori* Eradication before IBD onset (%)	4 (3)	18 (7)	0.16	0.43 (0.14-1.29)

3.4 UC patients *vs.* CD patients

3.4.1 Epidemiological data

Based on the histological type of IBD we divided the 127 IBD patients in two groups: UC and CD. Ninety of the 127 enrolled IBD patients had CD (34 M: 56 F, mean age 41.5±12.02 years, range 18-72 years). Thirty-seven IBD patients had UC (22 M: 15 F, mean age 42±11.98 years, range 18-67 years). No significant difference was found regarding gender and age of CD and UC patients.

The percentage of study participants that had no siblings was similar between CD and UC patients (18% *vs.* 22%, respectively, OR 0.78, 95% CI 0.3-2.02, p=0.78). Similarly, the percentage of patients that had less than three siblings did not differ between CD and UC (68% *vs*. 68%, respectively, OR 1, 95% CI 0.46-2.41, p=1.06). Finally, also the percentage of CD and UC patients reporting to have more than three siblings was similar (14% *vs*. 10%, OR 0.77%, 95% CI 0.38-4.22, p=1.62). A detailed description of the demographics data is reported in Table 5.

Table 5. Comparison of demographics data in patients with Crohn´s disease (CD) and ulcerative colitis (UC).

*: p< 0.05; °: Mann–Whitney U test for continuous data and by Fisher´s exact test for categorical variables; ᶜ: odds ratios (OR) with corresponding 95% confidence intervals.

	CD (90)	**UC (37)**	***p*°**	**OR (CI 95%)** ᶜ
Male/female	34/56	22/15	NS	
Median age ± Standard deviation (years)	41.5±12	40±11.9	NS	
Siblings (%)				
No siblings	16 (18)	8 (22)	0.62	0.78 (0.30-2.02)
< 3	62 (68)	25 (68)	1	1.06 (0.46-2.41)
≥ 3	12 (14)	4 (10)	0.77	1.62 (0.38- 4.22)

3.4.2 H. pylori serological status

Overall, 12% (11 out of 90) of patients with CD and 11% (4 out of 37) of patients with UC had a positive serology for *H. pylori*. Increased titers of anti- *H. pylori* -IgG, anti-CagA-IgG or both were observed respectively in 8 (9%), 3 (3%), 1 (1%) CD patients and 2 (5%), 3 (8%) and 1 (3%) UC patients. Seventeen out of 90 CD patients (19%) and 5/37 UC patients (13%) were *H. pylori*-positive (positive *H. pylori* serology and/or previous eradication therapy) (OR 1.4, 95% CI 0.5-4.3, p=0.6). Three out of 90 CD patients and 1 UC patient developed an IBD after *H. pylori* eradication therapy (OR 1.24, 95% CI 0.12-12.33, p=1). Detailed information on *H. pylori* serology and history of *H. pylori* eradication therapy in patients with CD and UC is reported in Table 6.

Table 6. Comparison of demographics data, *H. pylori* serology and history of *H. pylori* eradication therapy in patients with Crohn´s disease (CD) and ulcerative colitis (UC).

[a]: anti- *H. pylori* IgG titer >30 EIU; [b]: anti-CagA titer >6.25 U/mL; [c]: defined as positive anti- *H. pylori* IgG or positive anti-CagA or positive medical history for *H. pylori* eradication;*: p< 0.05; **°:** Mann–Whitney U test for continuous data and by Fisher´s exact test for categorical variables; **ᶜ**: odds ratios (OR) with corresponding 95% confidence intervals.

	CD (90)	**UC (37)**	***p*°**	**OR (CI 95%) ᶜ**
H. pylori positive (%) [a]	8 (9)	2 (5)	0.72	1.70 (0.34-8.45)
H. pylori CagA positive (%) [b]	3 (3)	3 (8)	0.35	0.39 (0.07-2.03)
H. pylori positive IgG and CagA (%)	1 (1)	1 (3)	0.49	0.40 (0.02-6.64)
H. pylori positive IgG and/or CagA (%)	11 (12)	4 (11)	1	1.14 (0.34-3.86)
H. pylori positive IgG and/or CagA and/or previous eradication therapy (%) [c]	17 (19)	5 (13)	0.6	1.4 (0.5-.4.3)
H. pylori eradication before IBD onset (%)	3 (3)	1 (3)	1	1.24 (0.12-12.33)

3.5 CD patients *vs.* control

3.5.1 Epidemiological data

We further performed a subgroup analysis considering CD patient and two sex- and age (±5 years) matched healthy blood donors (N=180). In this investigation, 79 (44%) out 180 controls were male and 101 (56%) female, median age 38.3±11.9 years. No significant difference was found regarding gender and age of CD patients and blood donors. The percentage of study participants that had no siblings was similar between CD patients and matched blood donors (18% vs. 22%, respectively, OR 0.78, 95% CI 0.40-1.49, p=0.52). Similarly, the percentage of study participants that had less than three siblings did not differ between CD patients and controls (68% vs. 65%, respectively, OR 1.19, 95% CI 0.69-2.04, p=0.58). Finally, also the percentage of CD patients and controls reporting to have more than three siblings was similar (14% vs. 13%, OR 1, 95% CI 0.47-2.10, p=1). A detailed description of the demographics data is reported in Table 7.

Table 7. Comparison of demographics data in patients with Crohn´s disease (CD) and controls.

*: p< 0.05; °: Mann–Whitney U test for continuous data and by Fisher´s exact test for categorical variables; ᶜ: odds ratios (OR) with corresponding 95% confidence intervals.

	CD (90)	**Controls (180)**	***p*°**	**OR (CI 95%) ᶜ**
Male/female	34/56	79/101	NS	
Median age ± Standard deviation (years)	41.5±12	38.3±11.9	NS	
Siblings (%)				
No siblings	16 (18)	39 (22)	0.52	0.78 (0.40-1.49)
< 3	62 (68)	117 (65)	0.58	1.19 (0.69-2.04)
≥ 3	12 (14)	24 (13)	1	1 (0.47-2.10)

3.5.2 H. pylori serological status

Overall, 11% (12 out of 90) of patients with CD had a positive serology for *H. pylori*. Increased titers of anti- *H. pylori* -IgG, anti-CagA-IgG or both were observed respectively in 8 (9%), 3 (3%) and 1 (1%) CD patients.

Seventeen (19%) out of 90 CD patients and 42/180 blood donor) were *H. pylori*-positive (positive *H. pylori* serology and/or previous eradication therapy) (OR 0.7, 95% CI 0.3-1.3, p=0.4).

An inverse association between *H. pylori* infection and CD (OR 0.4, 95% CI 0.19- 0.82, p<0.01) was observed. Detailed information on the statistical analysis is reported in Table 8.

Table 8. Comparison of *H. pylori* serology and history of *H. pylori* eradication therapy in patients with Crohn´s disease (CD) and controls.

[a]: anti- *H. pylori* IgG titer >30 EIU; [b]: anti-CagA titer >6.25 U/mL; [c]: defined as positive anti- *H. pylori* IgG or positive anti-CagA or positive medical history for *H .pylori* eradication;*: p< 0.05; **°:** Mann–Whitney U test for continuous data and by Fisher´s exact test for categorical variables; **ᶜ**: odds ratios (OR) with corresponding 95% confidence intervals.

	CD (90)	**Controls (180)**	***p*°**	**OR (CI 95%) ᶜ**
H. pylori positive (%) [a]	8 (9)	43 (24)	0.004*	0.33 (0.14-0.74)
H. pylori CagA positive (%) [b]	3 (3)	19 (10)	0.084	0.29 (0.08-1.01)
H. pylori positive IgG and CagA (%)	1 (1)	18 (10)	0.009*	0.10 (0.01- 0.81)
H. pylori positive IgG and/or CagA (%)	11 (12)	46 (25)	0.01*	0.4 (0.19-0.82)
H. pylori positive IgG and/or CagA and/or previous eradication therapy (%) [c]	17 (19)	42 (23)	0.4	0.7 (0.3-1.3)

3.6 UC patients *vs.* control

3.6.1 Epidemiological data

Finally, we performed a subgroup analysis considering UC patient and two sex- and age (±5 years) matched healthy blood donors (N=74). In this investigation, 44 (59%) out 74 controls were male and 30 (41%) female, median age 42.8±12.0 years. No significant difference was found regarding gender and age of UC patients and blood donors. The percentage of study participants that had no siblings was similar between UC patients and matched blood donors (22% *vs.* 18%, respectively, OR 1.29, 95% CI 0.48- 3.46, p=0.61). Similarly, the percentage of study participants that had less than three siblings did not differ between UC patients and controls (68% vs. 63%, respectively, OR 1.19, 95% CI 0.51- 2.75, p=0.83). Finally, also the percentage of CD patients and controls reporting to have more than three siblings was similar (10% vs. 19%, OR 0.51, 95% CI 0.15- 1.7, p=0.41). A detailed description of the demographics data is reported in Table 9.

Table 9. Comparison of demographics data in patients with ulcerative colitis (UC) and controls.

*: p< 0.05, °: Mann–Whitney U test for continuous data and by Fisher´s exact test for categorical variables; ᶜ: odds ratios (OR) with corresponding 95% confidence intervals.

	UC (37)	**Controls (74)**	***p*°**	**OR (CI 95%)** ᶜ
Male/female	22/15	44/30	NS	
Median age ± Standard deviation (years)	40±11.9	42.8±12.0	NS	
Siblings (%)				
No siblings	8 (22)	13 (18)	0.61	1.29 (0.48-3.46)
< 3	25 (68)	47 (63)	0.83	1.19 (0.51-2.75)
≥ 3	4 (10)	14 (19)	0.41	0.51 (0.15-1.7)

3.6.2 H. pylori serological status

Overall, 5% (2 out of 37) of patients with UC had a positive serology for *H. pylori*. Increased titers of anti- *H. pylori* -IgG, anti-CagA-IgG or both were observed respectively in 4 (11%), 3 (8%) and 1 (3%) UC patients. Five (13%) out of 37 UC patients and 20/74 blood donors were *H. pylori*-positive (positive *H. pylori* serology and/or previous eradication therapy) (OR 0.4, 95% CI 0.1-1.2, p=0.1). An inverse association between *H. pylori* infection and UC (OR 0.19, 95% CI 0.04- 0.87, p<0.03) was observed. Detailed information on the statistical analysis is reported in Table 10.

Table 10. Comparison of demographics data, *H. pylori* serology and history of *H. pylori* eradication therapy in patients with ulcerative colitis (UC) and controls.
[a]: anti- *H. pylori* IgG titer >30 EIU; [b]: anti-CagA titer >6.25 U/mL; [c]: defined as positive anti- *H. pylori* IgG or positive anti-CagA or positive medical history for *H. pylori* eradication ;*: p< 0.05, °: Mann–Whitney U test for continuous data and by Fisher´s exact test for categorical variables; [ç]: odds ratios (OR) with corresponding 95% confidence intervals.

	UC (37)	**Controls (74)**	***p*°**	**OR (CI 95%) [ç]**
H. pylori positive (%) [a]	2 (5)	17 (23)	0.03*	0.19 (0.04- 0.87)
H. pylori CagA positive (%) [b]	3 (8)	8 (11)	0.74	0.72 (0.18-2.92)
H. pylori positive IgG and CagA (%)	1 (3)	6 (8)	0.42	0.31 (0.03-2.71)
H. pylori positive IgG and/or CagA (%)	4 (11)	19 (26)	0.08	0.35 (0.1-1.12)
H. pylori positive IgG and/or CagA and/or previous eradication therapy (%) [c]	5 (13)	20 (27)	0.1	0.4 (0.1-1.2)

3.7 Previous *H. pylori* eradication

The rate of previous *H. pylori* eradication therapy in patients who thereafter developed an IBD was lower but not statistically different from that observed in the control group (3% and 7%, respectively, OR 0.43, 95% CI 0.14-1.29, p=0.16). Only four out of 127 patients developed an IBD (3 CD and 1 UC) after *H. pylori* eradication therapy. Detailed records of these four patients are reported in Table 11. Indications for *H. pylori* testing and eradication therapy previous to IBD onset were epigastric pain (N=2), bloating (N=1) and heartburn (N=1). In three out of 4 patients *H. pylori* gastritis was diagnosed by esophagogastroduodenoscopy with biopsies. In one patient the general practitioner made the diagnosis of *H. pylori* infection by serology (not in line with guidelines). In all four patients eradication therapy was prescribed by their general practitioner. The latency between *H. pylori* eradication therapy and IBD onset was of 6 to 12 months.

IBD patients and controls with previous *H. pylori* infection were eradicated according to the current German [66] and European guidelines [91] [67] and to the local clarithromycin resistance rate (less than 15%) [92]. In particular, all controls and all but 2 IBD patients were eradicated before 2016 and received a clarithromycin-based triple therapy for 7 days, whereas 2 IBD patients received eradication in 2016 and 2017 with a 14-day clarithromycin-based triple therapy and a 10-day quadruple therapy with Pylera® and omeprazole, respectively.

A successful eradication was documented by a ^{13}C-urea breath test or *H. pylori* stool antigen test in all study participants. Detailed information on demographic and clinical data of the IBD patients is reported in Table 11.

Table 11. Epidemiological and clinical data of the 4 patients that developed an inflammatory bowel disease (IBD) after *H. pylori* eradication therapy.

*: Symptoms before the *H. pylori* diagnosis; EGD: esophagogastroduodenoscopy; PAC: Clarithromycin 500 mg bid, Amoxicillin 1g bid and Pantoprazole 40 mg bid; PMC: Clarithromycin 500 mg bid, Metronidazol 400 bid and Pantoprazole 40 mg bid; UC: ulcerative colitis; CD: Crohn´s disease; [1]Thiopurines: Azathioprine or 6-mercaptopurine.

	Patient 1	***Patient 2***	***Patient 3***	***Patient 4***
Gender	female	male	female	male
Age at diagnosis of *H. pylori* infection (years)	43	34	41	33
Symptoms*	epigastric pain	epigastric pain	bloating	heartburn
H. pylori testing	EGD	EGD	EGD	Serology
Histological findings	chronic-active gastritis	chronic-active gastritis	chronic-active gastritis	-
H. pylori eradication therapy	7-day PAC	7-day PMC	7-day PAC	7-dayPAC
Successfully eradication	yes	yes	yes	yes
IBD Diagnosis	CD	UC	CD	CD
IBD localization	ileum	left colon	colon	ileum
Latency between *H. pylori* eradication and IBD Diagnosis	6 months	8 months	12 months	12 months
Biological therapy	Thiopurines[1] +Infliximab	Infliximab	Thiopurines[1] +Infliximab	Infliximab

4. DISCUSSION

To the best of our knowledge, this is the first prospective case-control study exploring the association of *H. pylori* eradication therapy with the onset of IBD.
In our study IBD onset was not associated with a previous *H. pylori* eradication therapy.
However, four of our IBD patients received *H. pylori* eradication therapy prior to definitive IBD diagnosis. The hypothesis that profound changes of gut microbiota composition induced by *H. pylori* eradication therapy (two antibiotics plus a PPI) may contribute, in a subset of individuals and under certain circumstances, to the development of IBD is fascinating and supported by experimental data.

4.1 Role of antibiotics on IBD onset

A previous therapy including 2 antibiotics and a PPI for *H. pylori* eradication was not associated with an increased the risk of developing IBD in the current study. According to published literature, the relationship between exposure to antibiotics and the risk of developing IBD is still unclear [93–95].
Metagenomic studies demonstrated an uneven recovery of the human gut microbiome after treatment with antibiotics [96–98]. Furthermore, in a subset of subjects with lower initial microbiome diversity, recovery of gut microbiome 3 months after antibiotic therapy was characterized by enrichment in opportunistic pathogens [99].
In line with the 3 case reports available in the literature, the time frame for the development of IBD after eradication therapy was 6-12 months also in our four patients. This finding was also consistent with the results of a population-based case-control study that used the Rochester Epidemiology Project of Olmsted County (Minnesota) [87]. In this study the use of antibiotics was associated with an increased risk of developing both new-onset CD and UC and the risk was highest in the first year after antibiotic intake.

One may speculate that "predisposed" subjects with lower initial microbiome diversity who receive antibiotic therapy may require at least 6-12 months for developing a clinically evident IBD. Thus, assessing prospectively microbiota prior and subsequent to *H. pylori* eradication therapy might be helpful for understanding the role of microbiota in relation to IBD onset.

The regimen used for *H. pylori* eradication therapy may play a role for the hypothesized IBD risk. In particular, all the reported IBD cases developed following a clarithromycin-based triple therapy, whereas no cases of new-onset IBD have been described after quadruple bismuth-based eradication therapy. Indeed, at least for what metronidazole and the risk of IBD concerns, available data are inconsistent [87].

4.2 Role of *H. pylori* infection on IBD onset

Although the etiology of IBD is poorly understood [88], there is an increasing evidence that environmental factors operating early in life can influence the risk of develop IBD many years later.

The hygiene hypothesis argued that the exposure to infections in early stage of life decreases the risk of developing autoimmune disorders late in life [100]. First Gent [101] and after MacDonald [102] suggested that a lack of exposures to enteric pathogens, especially early in life, leads to CD susceptibility in a genetically susceptible host.

Hence, multiple childhood infections and poor hygiene have been suggested as protective, by allowing the host to develop tolerance or immunity to agents that could trigger the disease at a later time. Excessive sanitation might limit exposure to environmental antigens and impair the functional maturation of the mucosal immune system and induction of immune tolerance, which ultimately results in inappropriate immune responses when re-exposed to these antigens later in life [100].

Various studies consistently have shown a low *H. pylori* seroprevalence in patients with IBD, suggesting a protective role of this infection in the

development of IBD [103]. Also in our study, we found a negative inverse association between *H. pylori* infection and IBD. However, is worth to underline that all our IBD patients were under a biological therapy. It is unclear whether the negative association of *H. pylori* seroprevalence and IBD is confounded by other variables, such as immunosuppressive therapy or environmental factors. This aspect is currently under scrutiny at our site.

Epidemiological studies in human populations have documented an inverse association between *H. pylori* infection and the risk of developing allergic disease as well [104]. The postulated protective effect of *H. pylori* infection against allergic diseases and possibly IBD may be mediated by regulatory T cells. Indeed, *H. pylori* is typically acquired during early childhood and the immunological tolerance towards *H. pylori* is driven by inducible regulatory T cells which are also required for the suppression of allergen-specific immune responses [105].

On the other hand, also the loss of *H. pylori* induced immune response may play a role in increasing the susceptibility to develop IBD as well. In an animal model of mongolian gerbils [106], *H. pylori* infection induced a microbiota shift that became exclusively overt in the large intestinal tract although no histopathological changes of the intestinal mucosa were detected. Hence, loss of *H. pylori* induced alteration of the stomach might trigger large intestinal microbiota changes predisposing to the development of IBD.

The rate of patients receiving *H. pylori* eradication therapy before IBD onset in our study was very low and less than half of the rate observed in the control group. Thus, on a population scale, the benefit deriving from the cure of *H. pylori* infection overwhelms the hypothetical risk of developing an IBD.

4.1 Role of *H. pylori* CagA on IBD onset

The data on the prevalence of virulent *H. pylori* strains in IBD patients are limited. Wagtmans et al. [107] showed that the majority (66%) of *H. pylori* seropositive patients with CD were infected by *H. pylori* CagA (+) strains although a similar proportion of controls (69.4%) were also infected by these

strains. Recently, Lord et al. [108] reported that the inverse association of *H. pylori* with IBD was restricted to the CagA-positive strain and observed a protective effect of CagA only on CD. In our study, a negative inverse association between CagA status and IBD could not be confirmed, possibly as a consequence of the lower number of IBD patients included.

The mechanism underlying the inverse association between cagA-positive *H. pylori* strains and the lower incidence of CD is still unclear. It has been proposed that the host immune response to CagA-positive *H. pylori* strains may alter the Th1- and Th2-type immune responses with subsequent induction of immunoregulatory lymphocytes that might induce IBD [109].

4.2 Strengths and limitations of the study

Strength of our study is the selection of controls from a well-characterized cohort of blood donors from the same region (Saxony-Anhalt, Germany) as the IBD group and tested both for the anti-*H. pylori* IgG in general and specific for the CagA antigen. In addition, in both IBD and control groups, information on previous *H. pylori* treatment was retrieved.

A limitation of the present study is the missing data on prior antibiotic use. However, this information is not reliable when collected on a patient´s history basis and we had no access to the prescription data of physicians who took care of our patients prior to IBD diagnosis. Whether previous antibiotic treatments may have increased the susceptibility of our patients to develop IBD after *H. pylori* eradication therapy cannot be excluded. Based on previous case-reports and population-based studies we excluded controls who received eradication therapy less than 1 year before enrollment. As an increased risk of developing IBD may persist till up to 5 years after antibiotic treatment, the development of an IBD in our controls later on cannot be excluded.

Another limitation is the indirect detection of *H. pylori* by serology. After a successful eradication of *H. pylori* infection, a seroconversion may take several months, or even years, and thus the possibility of false positive results should be

taken into account [110]. Studies investigating the presence of the bacterium by direct tests as well as the effect of active *H. pylori* infection on the risk of development of IBD may provide more precise estimates.

5. CONCLUSIONS

In our study population, previous *H. pylori* eradication therapy was not associated with an increased risk of developing IBD. Our data should reassure physicians when prescribing *H. pylori* eradication therapy.

Further studies are warranted to address the question as to whether *H. pylori* eradication therapy (the loss of *H. pylori* induced immune response or the combination of two antibiotics and a proton pump inhibitor) may trigger IBD development in a specific subgroup of patients.

6. REFERENCES

1 Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *The Lancet* 369(9573), 1627–1640 (2007).

2 Kalla R, Ventham NT, Satsangi J, Arnott IDR. Crohn's disease. *BMJ* 349(13), g6670-g6670 (2014).

3 Adams SM, Bornemann PH. Ulcerative colitis. *American family physician* 87(10), 699–705 (2013).

4 Ng SC, Shi HY, Hamidi N *et al.* Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet (London, England)* 390(10114), 2769–2778 (2017).

5 Kappelman M., Rifas-Shiman S., Kleinman K, Ollendorf D., Bousvaros A., Grand R. and Finkelstein J. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. *Clin Gastroenterol Hepatol* 5, 1424-1429 (2007).

6 Burisch J, Jess T, Martinato M, Lakatos PL. The burden of inflammatory bowel disease in Europe. *Journal of Crohn's and Colitis* 7(4), 322–337 (2013).

7 Johnston R, Logan R. What is the peak age for onset of IBD? *Inflamm Bowel Dis* 15(9), pp. 1438-1447 (2009).

8 Karlinger K, Györke T, Makö E, Mester A, Tarján Z. The epidemiology and the pathogenesis of inflammatory bowel disease. *European journal of radiology* 35(3), 154–167 (2000).

9 Gajendran M, Loganathan P, Catinella AP, Hashash JG. A comprehensive review and update on Crohn's disease. *Disease-a-month : DM* 64(2), 20–57 (2018).

10 Naser SA, Arce M, Khaja A *et al.* Role of ATG16L, NOD2 and IL23R in Crohn's disease pathogenesis. *World journal of gastroenterology* 18(5), 412–424 (2012).

11 Khasawneh M, Spence AD, Addley J, Allen PB. The role of smoking and alcohol behaviour in the management of inflammatory bowel disease. *Best practice & research. Clinical gastroenterology* 31(5), 553–559 (2017).

12 Kuenzig ME, Lee SM, Eksteen B *et al.* Smoking influences the need for surgery in patients with the inflammatory bowel diseases: a systematic review and meta-analysis incorporating disease duration. *BMC gastroenterology* 16(1), 143 (2016).

13 Ananthakrishnan A, Higuchi L, Huang E *et al.* Aspirin, nonsteroidal antiinflammatory drug use, and risk for Crohn disease and ulcerative colitis: a cohort study. *Ann Intern Med* 156, 350-359 (2012).

14 Bernstein CN. The Brain-Gut Axis and Stress in Inflammatory Bowel Disease. *Gastroenterology clinics of North America* 46(4), 839–846 (2017).

15 Cámara R, Schoepfer A, Pittet V, Begré S, Känel R von. Mood and nonmood components of perceived stress and exacerbation of Crohn's disease. *Inflamm Bowel Dis* 17, 2358-2365 (2011).

16 Abegunde AT, Muhammad BH, Bhatti O, Ali T. Environmental risk factors for inflammatory bowel diseases: Evidence based literature review. *World journal of gastroenterology* 22(27), 6296–6317 (2016).

17 Kostic A, Xavier R, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* 146(6), 1489–1499 (2014).

18 Shen Z, Zhu C, Quan Y *et al*. Update on intestinal microbiota in Crohn's disease 2017: Mechanisms, clinical application, adverse reactions, and outlook. *J Gastroenterol Hepatol* 32(11), 1804–1812 (2017).

19 Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clinical journal of gastroenterology* 11(1), 1–10 (2018).

20 Gálvez J. Role of Th17 Cells in the Pathogenesis of Human IBD. *ISRN inflammation* 2014, 928461 (2014).

21 Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 140(6), 1756–1767 (2011).

22 Magro F, Gionchetti P, Eliakim R *et al.* Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. *Journal of Crohn's and Colitis* 11(6), 649–670 (2017).

23 Gomollón F, Dignass A, Annese V *et al.* 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: Part 1: Diagnosis and Medical Management. *Journal of Crohn's and Colitis* 11(1), 3–25 (2017).

24 Laube R, Liu K, Schifter M, Yang J, Suen M, Leong R. Oral and upper gastrointestinal Crohn's disease. *J Gastroenterol Hepatol* 33(2), 355–364 (2018).

25 Panés J, Rimola J. Perianal fistulizing Crohn's disease: pathogenesis, diagnosis and therapy. *Nature reviews. Gastroenterology & hepatology* 14(11), 652–664 (2017).

26 Vavricka S, Schoepfer A, Scharl M, Lakatos P, Navarini A, Rogler G. Extraintestinal Manifestations of Inflammatory Bowel Disease. *Inflamm Bowel Dis* 21(8), 1982–1992 (2015).

27 Shi HY, Ng SC. The state of the art on treatment of Crohn's disease. *J Gastroenterol* 53(9), 989–998 (2018).

28 Vermeire S, Schreiber S, Sandborn WJ, Dubois C, Rutgeerts P. Correlation between the Crohn's disease activity and Harvey-Bradshaw indices in assessing Crohn's disease severity. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 8(4), 357–363 (2010).

29 Narula N, Alshahrani A-A, Yuan Y, Reinisch W, Colombel J-F. Patient-Reported Outcomes and Endoscopic Appearance of Ulcerative Colitis-a Systematic Review and Meta-Analysis. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* doi:10.1016/j.cgh.2018.06.015 (2018) (Epub ahead of print) .

30 Peyrin-Biroulet L, Panés J, Sandborn WJ *et al.* Defining Disease Severity in Inflammatory Bowel Diseases: Current and Future Directions. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 14(3), 348-354.e17 (2016).

31 Woodman I, Schofield JB, Haboubi N. The histopathological mimics of inflammatory bowel disease: a critical appraisal. *Techniques in coloproctology* 19(12), 717–727 (2015).

32 Pai RK, Geboes K. Disease activity and mucosal healing in inflammatory bowel disease: a new role for histopathology? *Virchows Archiv : an international journal of pathology* 472(1), 99–110 (2018).

33 Sood A, Midha V, Singh A. Differential diagnosis of Crohn's disease versus ileal tuberculosis. *Current gastroenterology reports* 16(11), 418 (2014).

34 Matsuoka K, Kobayashi T, Ueno F *et al.* Evidence-based clinical practice guidelines for inflammatory bowel disease. *J Gastroenterol* 53(3), 305–353 (2018).

35 Sands BE. Biomarkers of Inflammation in Inflammatory Bowel Disease. *Gastroenterology* 149(5), 1275-1285.e2 (2015).

36 Ward MG, Kariyawasam VC, Mogan SB *et al.* Prevalence and Risk Factors for Functional Vitamin B12 Deficiency in Patients with Crohn's Disease. *Inflammatory bowel diseases* 21(12), 2839–2847 (2015).

37 Walsham N, Sherwood R. Fecal calprotectin in inflammatory bowel disease. *Clin Exp Gastroenterol* 9, 21–29 (2016).

38 Gecse KB, Vermeire S. Differential diagnosis of inflammatory bowel disease: imitations and complications. *The Lancet Gastroenterology & Hepatology* 3(9), 644–653 (2018).

39 Magro F, Langner C, Driessen A *et al.* European consensus on the histopathology of inflammatory bowel disease. *Journal of Crohn's & colitis* 7(10), 827–851 (2013).

40 Bryant RV, Friedman AB, Wright EK *et al.* Gastrointestinal ultrasound in inflammatory bowel disease: an underused resource with potential paradigm-changing application. *Gut* 67(5), 973–985 (2018).

41 Maconi G, Nylund K, Ripolles T *et al.* EFSUMB-Empfehlungen und klinische Leitlinien für den gastrointestinalen Ultraschall (GIUS) chronisch entzündlichen Darmerkrankungen (CED). *Ultraschall in der Medizin* 39(3), 304–317 (2018).

42 Liu W, Liu J, Xiao W, Luo G. A Diagnostic Accuracy Meta-analysis of CT and MRI for the Evaluation of Small Bowel Crohn Disease. *Academic radiology* 24(10), 1216–1225 (2017).

43 Bouchard D, Abramowitz L, Bouguen G *et al.* Anoperineal lesions in Crohn's disease: French recommendations for clinical practice. *Techniques in coloproctology* 21(9), 683–691 (2017).

44 Siegel CA. Refocusing IBD Patient Management: Personalized, Proactive, and Patient-Centered Care. *Am J Gastroenterol* doi:10.1038/s41395-018-0246-x (2018) (Epub ahead of print) .

45 Shen B. IBD: Step-up vs top-down therapy for Crohn's disease: medicine vs surgery. *Nat Rev Gastroenterol Hepatol* 14(12), 693–695 (2017).

46 Harbord M, Eliakim R, Bettenworth D *et al.* Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 2: Current Management. *Journal of Crohn's and Colitis* 11(7), 769–784 (2017).

47 Gionchetti P, Dignass A, Danese S *et al.* 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: Part 2: Surgical Management and Special Situations. *Journal of Crohn's and Colitis* 11(2), 135–149 (2017).

48 Hooi JKY, Lai WY, Ng WK *et al.* Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. *Gastroenterology* 153(2), 420–429 (2017).

49 Tsipotis E, Leontiadis GI. Editorial: the prevalence of *Helicobacter pylori* infection worldwide-knowns and unknowns. *Alimentary pharmacology & therapeutics* 47(9), 1330–1331 (2018).

50 Franck C, Hoffmann A, Link A *et al.* Prevalence of *Helicobacter pylori* infection among blood donors in Saxony-Anhalt, Germany - a region at intermediate risk for gastric cancer. *Z Gastroenterol* 55(7), 653–656 (2017). dx.doi.org/10.1055/s-0043-106311.

51 Kalach N, Bontems P, Raymond J. *Helicobacter pylori* infection in children. *Helicobacter* 22 Suppl 1 (2017).

52 Waskito LA, Salama NR, Yamaoka Y. Pathogenesis of *Helicobacter pylori* infection. *Helicobacter* 23 Suppl 1, e12516 (2018).

53 Amieva MR, Vogelmann R, Covacci A, Tompkins LS, Nelson WJ, Falkow S. Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA. *Science (New York, N.Y.)* 300(5624), 1430–1434 (2003).

54 Chang W-L, Yeh Y-C, Sheu B-S. The impacts of *H. pylori* virulence factors on the development of gastroduodenal diseases. *Journal of biomedical science* 25(1), 68 (2018).

55 Tegtmeyer N, Neddermann M, Asche CI, Backert S. Subversion of host kinases: a key network in cellular signaling hijacked by *Helicobacter pylori* CagA. *Molecular microbiology* 105(3), 358–372 (2017).

56 Kim I-J, Blanke SR. Remodeling the host environment: modulation of the gastric epithelium by the *Helicobacter pylori* vacuolating toxin (VacA). *Frontiers in cellular and infection microbiology* 2, 37 (2012).

57 Chauhan N, Tay ACY, Marshall BJ, Jain U. *Helicobacter pylori* VacA, a distinct toxin exerts diverse functionalities in numerous cells: An overview. *Helicobacter* doi:10.1111/hel.12544 (2018) (Epub ahead of print) .

58 Utsch C, Haas R. VacA's Induction of VacA-Containing Vacuoles (VCVs) and Their Immunomodulatory Activities on Human T Cells. *Toxins* 8(6) (2016).

59 Palframan SL, Kwok T, Gabriel K. Vacuolating cytotoxin A (VacA), a key toxin for *Helicobacter pylori* pathogenesis. *Frontiers in cellular and infection microbiology* 2, 92 (2012).

60 Kumar S, Dhiman M. Inflammasome activation and regulation during *Helicobacter pylori* pathogenesis. *Microbial pathogenesis* 125, 468–474 (2018).

61 Jonaitis L, Pellicano R, Kupcinskas L. *Helicobacter pylori* and nonmalignant upper gastrointestinal diseases. *Helicobacter* 23 Suppl 1, e12522 (2018).

62 Graham DY. History of *Helicobacter pylori,* duodenal ulcer, gastric ulcer and gastric cancer. *World journal of gastroenterology* 20(18), 5191–5204 (2014).

63 Herrero R, Park JY, Forman D. The fight against gastric cancer - the IARC Working Group report. *Best practice & research. Clinical gastroenterology* 28(6), 1107–1114 (2014).

64 Venerito M, Vasapolli R, Rokkas T, Malfertheiner P. Gastric cancer: epidemiology, prevention, and therapy. *Helicobacter* 23 Suppl 1, e12518 (2018).

65 Floch P, Mégraud F, Lehours P. *Helicobacter pylori* Strains and Gastric MALT Lymphoma. *Toxins* 9(4) (2017).

66 Fischbach W, Malfertheiner P, Lynen Jansen P *et al.* S2k-Guideline *Helicobacter pylori* and gastroduodenal ulcer disease. *Z Gastroenterol* 54(4), 167–206 (2016).

67 Malfertheiner P, Megraud F, O'Morain CA *et al.* Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut* 66(1), 6–30 (2017).

68 Tonkic A, Vukovic J, Vrebalov Cindro P, Pesutic Pisac V, Tonkic M. Diagnosis of *Helicobacter pylori* infection : A short review. *Wiener klinische Wochenschrift* 130(17-18), 530–534 (2018).

69 Burucoa C, Delchier J-C, Courillon-Mallet A *et al.* Comparative evaluation of 29 commercial *Helicobacter pylori* serological kits. *Helicobacter* 18(3), 169–179 (2013).

70 Malfertheiner P. Diagnostic methods for *H. pylori* infection: Choices, opportunities and pitfalls. *United European gastroenterology journal* 3(5), 429–431 (2015).

71 Lee JY, Kim N. Diagnosis of *Helicobacter pylori* by invasive test: histology. *Annals of translational medicine* 3(1), 10 (2015).

72 Rugge M, Meggio A, Pravadelli C *et al.* Gastritis staging in the endoscopic follow-up for the secondary prevention of gastric cancer: a 5-year prospective study of 1755 patients. *Gut* doi:10.1136/gutjnl-2017-314600 (2018) (Epub ahead of print) .

73 Flores-Treviño S, Mendoza-Olazarán S, Bocanegra-Ibarias P, Maldonado-Garza HJ, Garza-González E. *Helicobacter pylori* drug resistance: therapy changes and challenges. *Expert review of gastroenterology & hepatology* 12(8), 819–827 (2018).

74 Megraud F, Coenen S, Versporten A *et al. Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut* 62(1), 34–42 (2013).

75 Sonnenberg A. Review article: historic changes of *Helicobacter pylori*-associated diseases. *Alimentary pharmacology & therapeutics* 38, 329–342 (2013).

76 Thomson JM, Hansen R, Berry SH *et al.* Enterohepatic helicobacter in ulcerative colitis: potential pathogenic entities? *PloS one* 6(2), e17184 (2011).

77 Laharie D, Asencio C, Asselineau J *et al.* Association between enterohepatic *Helicobacter* species and Crohn's disease: a prospective cross-sectional study. *Aliment Pharmacol Ther,* 30, 283-293 (2009).

78 Lankarani KB, Honarvar B, Athari SS. The Mechanisms Underlying *Helicobacter Pylori*-Mediated Protection against Allergic Asthma. *Tanaffos* 16(4), 251–259 (2017).

79 el-Omar E, Penman I, Cruikshank G *et al.* Low prevalence of *Helicobacter pylori* in inflammatory bowel disease: association with sulphasalazine. *Gut* 35(10), 1385–1388 (1994).

80 Guslandi M, Fanti L, Testoni P. *Helicobacter pylori* seroprevalence in Crohn's disease: Lack of influence by pharmacological treatment. *Hepatogastroenterology* 49(47), 1296–1297 (2002).

81 Tursi A. Onset of Crohn's Disease after *Helicobacter pylori* Eradication. *Inflamm Bowel Dis* 12(10), 1008–1009 (2006).

82 Chiba M, Tsuji T, Takahashi K, Komatsu M, Sugawara T, Ono I. Onset of Ulcerative Colitis after *Helicobacter pylori* Eradication Therapy: A Case Report. *The Permanente journal* 20(2), e115-8 (2016).

83 Jovanovic I, Milosavjevic T, Jankovic G *et al.* Clinical onset of the Crohn's disease after eradication therapy of Helicobacter pylori infection. Does *Helicobacter pylori* infection interact with natural history of inflammatory bowel diseases? *Med Sci Monit* 7(1), 137–141 (2001).

84 Boyapati R, Satsangi J, Tzer Ho G. Pathogenesis of Crohn's disease. *F1000Prime Rep* 7 (2015).

85 Schulz C, Schütte K, Koch N *et al.* The active bacterial assemblages of the upper GI tract in individuals with and without Helicobacter infection. *Gut* 67(2), 216–225 (2018).

86 Sheehan D, Shanahan F. The gut microbiota in inflammatory bowel disease. *Gastroenterol Clin North Am* 46, 143–154 (2017).

87 Aniwan S, Tremaine WJ, Raffals LE, Kane SV, Loftus EV. Antibiotic Use and New-Onset Inflammatory Bowel Disease in Olmsted County, Minnesota: A Population-Based Case-Control Study. *Journal of Crohn's and Colitis* 12(2), 137–144 (2018).

88 Su H-J, Chiu Y-T, Chiu C-T *et al.* Inflammatory bowel disease and its treatment in 2018: Global and Taiwanese status updates. *Journal of the Formosan Medical Association = Taiwan yi zhi* doi:10.1016/j.jfma.2018.07.005 (2018) (Epub ahead of print) .

89 Rokkas T, Gisbert JP, Niv Y, O'Morain C. The association between *Helicobacter pylori* infection and inflammatory bowel disease based on meta-analysis. *United European gastroenterology journal* 3(6), 539–550 (2015).

90 WMA. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA* 310(20), 2191–2194 (2013).

91 Malfertheiner P, Megraud F, O'Morain CA *et al.* Management of *Helicobacter pylori* infection--the Maastricht IV/ Florence Consensus Report. *Gut* 61(5), 646–664 (2012).

92 Selgrad M, Meissle J, Bornschein J *et al.* Antibiotic susceptibility of *Helicobacter pylori* in central Germany and its relationship with the number of eradication therapies. *European journal of gastroenterology & hepatology* 25(11), 1257–1260 (2013).

93 Shaw S, Blanchard J, Bernstein C. Association between the use of antibiotics antibiotics and new diagnoses of Crohn's disease and ulcerative colitis. *Am J Gastroenterol* 106(12), 2133–2142 (2011).

94 Card T, Logan R, Rodrigues L, Wheeler J. Antibiotic use and the development of Crohn's disease. *Gut* 53, 246–250 (2004).

95 Ungaro R, Bernstein CN, Gearry R *et al.* Antibiotics Associated With Increased Risk of New-Onset Crohn's Disease But Not Ulcerative Colitis: A Meta-Analysis. *Am J Gastroenterol* 109(11), 1728–1738 (2014).

96 Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proceedings of the National Academy of Sciences of the United States of America* 108(1), 4554–4561 (2011).

97 Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS biology* 6(11), e280 (2008).

98 Rashid M-U, Rosenborg S, Panagiotidis G, Löfdal KS, Weintraub A, Nord CE. Ecological effect of ceftazidime/avibactam on the normal human intestinal microbiota. *International journal of antimicrobial agents* 46(1), 60–65 (2015).

99 Raymond F, Ouameur AA, Déraspe M *et al.* The initial state of the human gut microbiome determines its reshaping by antibiotics. *The ISME journal* 10(3), 707–720 (2016).

100 Cholapranee A, Ananthakrishnan AN. Environmental Hygiene and Risk of Inflammatory Bowel Diseases: A Systematic Review and Meta-analysis. *Inflammatory bowel diseases* 22(9), 2191–2199 (2016).

101 Gent AE, Hellier MD, Grace RH, Swarbrick ET, Coggon D. Inflammatory bowel disease and domestic hygiene in infancy. *Lancet (London, England)* 343(8900), 766–767 (1994).

102 MacDonald TT. Breakdown of tolerance to the intestinal bacterial flora in inflammatory bowel disease (IBD). *Clinical and experimental immunology* 102(3), 445–447 (1995).

103 van der Sloot KWJ, Amini M, Peters V, Dijkstra G, Alizadeh BZ. Inflammatory Bowel Diseases: Review of Known Environmental Protective and Risk Factors Involved. *Inflammatory bowel diseases* 23(9), 1499–1509 (2017).

104 Blaser M, Chen Y, Reibman J. Does *Helicobacter pylori* protect against asthma and allergy? *Gut* 57(5), 561–567 (2008).

105 Robinson K. *Helicobacter pylori*-Mediated Protection against Extra-Gastric Immune and Inflammatory Disorders: The Evidence and Controversies. *Diseases (Basel, Switzerland)* 3(2), 34–55 (2015).

106 Heimesaat MM, Fischer A, Plickert R *et al. Helicobacter pylori* induced gastric immunopathology is associated with distinct microbiota changes in the large intestines of long-term infected Mongolian gerbils. *PloS one* 9(6), e100362 (2014).

107 Wagtmans M, Witte A, Taylor D *et al.* Low seroprevalence of *Helicobacter pylori* antibodies in historical sera of patients with Crohn's disease. *Scand J Gastroenterol* 32, 712-718 (1997).

108 Lord AR, Simms LA, Hanigan K, Sullivan R, Hobson P, Radford-Smith GL. Protective effects of *Helicobacter pylori* for IBD are related to the cagA-positive strain. *Gut* 67(2), 393–394 (2018).

109 Chen Y, Blaser MJ. Inverse associations of *Helicobacter pylori* with asthma and allergy. *Archives of internal medicine* 167(8), 821–827 (2007).

110 Lee JH, Kim N, Chung JI *et al.* Long-term follow up of *Helicobacter pylori* IgG serology after eradication and reinfection rate of H. pylori in South Korea. *Helicobacter* 13(4), 288–294 (2008).

Annexes

Annex 1. Informed consent for IBD patients

OTTO – VON – GUERICKE – UNIVERSITÄT Magdeburg
Medizinische Fakultät
Zentrum für Innere Medizin
Klinik für Gastroenterologie, Hepatologie und Infektiologie
Direktor: Prof. Dr. med. Peter **Malfertheiner**
Leipziger Straße 44
D-39120 Magdeburg

Serumprävalenz von *Helicobacter pylori* im Rahmen der Studie: „Adaptive *Helicobacter pylori* - assoziierte bakterielle und Wirtssuszeptibilitätsfaktoren in der Magenkarzinogenese“

Liebe Patientin, lieber Patient,

an unserer Klinik wird derzeit eine klinische Studie zur Häufigkeit der Infektion mit dem Magenkeim *Helicobacter pylori* durchgeführt. Sie als Patienten mit chronischer entzündlicher Darmerkrankung können uns dabei wertvolle Informationen zur Häufigkeit des Keims liefern. Mit einer Teilnahme helfen Sie zukünftigen Patienten und erfahren, wenn Sie wünschen, ob bei Ihnen Kontakt mit *H. pylori* besteht.

Das Bakterium *Helicobacter pylori* verursacht bei einigen Menschen eine Infektion des Magens, die nur in wenigen Fällen mit Beschwerden einhergeht. Selten können jedoch als Spätfolge Geschwüre oder sogar Magenkrebs auftreten. Die Häufigkeit eines Kontaktes mit dem Erreger und der Krankheitsfall ist in Patienten mit chronischer entzündlicher Darmerkrankung nicht bekannt. Wir möchten deshalb die Antikörper gegen das Bakterium in Ihrem Blut bestimmen. Die Blutabnahme hierfür (1 Probenröhrchen; 8,5 ml) kann im Rahmen der ambulanten Kontrolle erfolgen. Der nachfolgende Fragebogen soll uns zusätzliche Informationen über die Krankheitshäufigkeit in verschiedenen Bevölkerungsgruppen ermöglichen.
Bei nachgewiesenem Kontakt mit *H. pylori* ist selbstverständlich eine medizinische Beratung und weitere Abklärung in unserer gastroenterologischen Ambulanz möglich.

Die Datenauswertung erfolgt anonym. Aus rechtlichen und ethischen Gründen benötigen wir für die Blutentnahme und wissenschaftliche Untersuchungen jedoch Ihr schriftliches Einverständnis.

Vielen Dank für Ihr Interesse. Über eine Teilnahme würden wir uns freuen.

Ort, Datum	Unterschrift Arzt	Unterschrift Proband

Kontakt:
Rosa Rosania
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Annex 2. Questionnaire for IBD patients

Serumprävalenz von *Helicobacter pylori* (HPPRE)

1. Alter in Jahren: _____

2. Geschlecht: ☐ männlich ☐ weiblich

3. Geburtsland: _____

4. Wo sind Sie aufgewachsen? ☐ Stadt ☐ Land

5. Anzahl der Geschwister: ☐ 0 ☐ 1 ☐ 2 ☐ mehr

6. Haben Sie Oberbauchbeschwerden? ☐ Nein ☐ Ja

7. Leiden Sie unter einer gesicherten Magenerkrankung? ☐ Nein ☐ Ja
   → Wenn ja, welche? _____

8. Nehmen Sie Medikamente ein, die die Magensäureproduktion hemmen (z. B. Pantozol®, Nexium® usw.)? ☐ Nein ☐ Ja

8. Sind bei Ihnen bösartige Erkrankungen des Magendarmtraktes in der Familie bekannt? ☐ Nein ☐ Ja
   → Wenn ja, welche? _____

9. Wurden Sie schon einmal gegen *Helicobacter pylori* behandelt? ☐ Nein ☐ Ja

   → Wenn ja, wann? _____

→ Mit welcher Antibiotikatherapie? _____

→ Von welchem Arzt? Name_____
Anschrift_____

10. Welche chronische entzündliche Darmerkrankung liegt bei Ihnen vor? ☐ Morbus Crohn ☐ Colitis Ulcerosa

11. Wann wurde Ihre chronische entzündliche Darmerkrankung erstmalig diagnostiziert? Jahr _____

12. Wurde Ihre chronische entzündliche Darmerkrankung nach der Einnahme von Antibiotikatherapie gegen *Helicobacter pylori* diagnostiziert? ☐ Ja ☐ Nein

Tel.-Nr. für Rückfragen: ***0391 / 67 13255*** oder ***13249***. Vielen Dank für Ihre Mühe !

Annex 3. Approval of the ethics committee

UNIVERSITÄTSKLINIKUM MAGDEBURG A.Ö.R. OTTO VON GUERICKE UNIVERSITÄT MAGDEBURG MEDIZINISCHE FAKULTÄT

Ethik-Kommission der Otto-von-Guericke-Universität an der Medizinischen Fakultät und am Universitätsklinikum Magdeburg A.ö.R.

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Datum
29.11.2016

Unser Zeichen: **80/11**

Adaptive Helicobacter pylori assoziierte genetische Marker und Wirtssuszeptibilitätsfaktoren in der Magenkarzinogenese

Sehr geehrter Herr Prof. Malfertheiner

zur vorstehend genannten Studie ist bei uns mit Schreiben vom 27.09.2016 ein Amendment – Rekrutierung einer zusätzlichen Vergleichsgruppe/Subgruppe, Blutentnahme, epidemiologiesche Basisinformationen - eingegangen.

Aus Ihren Ausführungen und nach Studium der Unterlagen lässt sich eine Änderung der Risiko-Nutzen-Lage zuungunsten einbezogener Patienten nicht ohne Weiteres ableiten, so dass nach Rücksprache mit dem Kommissionsvorsitzenden die Protokolländerungen bzw. -ergänzungen genehmigt werden.

Die zustimmende Bewertung – positives Votum - vom 13.07.2011 bleibt gültig.

Mit freundlichen Grüßen

Dr. med. N. Beck
Geschäftsführer der Ethik-Kommission

Ehrenerklärung (Declaration in German)

Ich erkläre, dass ich die der Medizinischen Fakultät der Otto-von-Guericke-Universität zur Promotion eingereichte Dissertation mit dem Titel

***Helicobacter pylori* eradication therapy is not associated with the onset of inflammatory bowel disease. A case-control study.**

in der Klinik für Gastroenterologie, Hepatologie und Infektiologie der Otto-von-Guericke-Universität Magdeburg mit Unterstützung durch PD Dr. med. Marino Venerito ohne sonstige Hilfe durchgeführt und bei der Abfassung der Dissertation keine anderen als die dort aufgeführten Hilfsmittel benutzt habe.

Bei der Abfassung der Dissertation sind Rechte Dritter nicht verletzt worden.

Ich habe diese Dissertation bisher an keiner in- oder ausländischen Hochschule zur Promotion eingereicht. Ich übertrage der Medizinischen Fakultät das Recht, weitere Kopien meiner Dissertation herzustellen und zu vertreiben.

Magdeburg, den

Erklärung zur strafrechtlichen Verurteilung

Ich erkläre hiermit, nicht wegen einer Straftat verurteilt worden zu sein, die Wissenschaftsbezug hat.

Magdeburg, den

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Curriculum vitae et studiorum

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01.04.2015	Assistenzarzt, Klinik für Gastroenterologie, Hepatologie und Infektiologie, *Otto-von-Guericke* Universität, Magdeburg, Direktor Prof. P. Malfertheiner
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Drittmittel, Preise

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Liste der wissenschaftlichen Publikationen, Stand 14.10.2018

Ierardi E, Hassan C, Zullo A, De Francesco V, Valle ND, Prencipe S, **Rosania R**, Morini S, Panella C. Segmental colitis associated with diverticula: a rare clinical entity and a new challenge for the gastroenterologist. *Dig Liver Dis*. 2009 Nov; 41(11):794-7

Ierardi E, Giorgio F, **Rosania R**, Zotti M, Prencipe S, Della Valle N, De Francesco V, Panella C. Mucosal assessment of tumor necrosis factor alpha levels on paraffined samples: a comparison between immunohistochemistry and real time polymerase chain reaction. *Scand J Gastroenterol*. 2010 Aug; 45(7-8):1007-8.

Ierardi E, Margiotta M, Giorgio F, **Rosania R**, Zotti M, Prencipe S, Prencipe S, delli Carri P, Greco P, Panella C. Tumor necrosis alpha serum levels parallels isolated hypertransaminasemia in the third trimester of pregnancy. *Ann Hepatol*. 2010 Apr-Jun; 9 (2):211-2.

Ierardi E, **Rosania R**, Zotti M, Principe S, Laonigro G, Giorgio F, de Francesco V, Panella C. Metabolic syndrome and gastro-esophageal reflux: A link towards a growing interest in developed countries. *World J Gastrointest Pathophysiol*. 2010 Aug 15; 1 (3):91-6.

Ierardi E, **Rosania R**, Zotti M, Giorgio F, Prencipe S, Valle ND, Francesco VD, Panella C. From chronic liver disorders to hepatocellular carcinoma: Molecular and genetic pathways. *World J Gastrointest Oncol*. 2010 Jun 15; 2 (6):259-64.

Ierardi E, Giorgio F, Zotti M, **Rosania R**, Principi M, Marangi S, Della Valle N, De Francesco V, Di Leo A, Ingrosso M, Panella C. Infliximab therapy downregulation of basic fibroblast growth factor/syndecan 1 link: a possible molecular pathway of mucosal healing in ulcerative colitis. *J Clin Pathol*. 2011 Nov; 64(11):968-72.

Francesco VD, Zullo A, Hassan C, Giorgio F, **Rosania R**, Ierardi E. Mechanisms of *Helicobacter pylori* antibiotic resistance: An updated appraisal. *World J Gastrointest Pathophysiol*. 2011 Jun 15; 2 (3):35-41.

Facciorusso A, Nacchiero MC, **Rosania R**, Laonigro G, Longo N, Panella C, Ierardi E. The use of human albumin for the treatment of ascites in patients with liver cirrhosis: item of safety, facts, controversies and perspectives. *Curr Drug Saf*. 2011 Sep 1; 6 (4):267-74.

Rosania R, Minenna MF, Giorgio F, Facciorusso A, De Francesco V, Hassan C, Panella C, Ierardi E. Probiotic multistrain treatment may eradicate *Helicobacter pylori* from the stomach of dyspeptics: a placebo-controlled pilot study. *Inflamm Allergy Drug Targets*. 2012 Jun; 11(3):244-9.

Facciorusso A, Nacchiero MC, **Rosania R**, Laonigro G, Giorgio F, Del Prete V, Panella C, Ierardi E. Pathways and gene expression profiles in hepatocellular carcinoma. *Minerva Gastroenterol Dieto*l. 2012 Mar; 58(1):35-48.

Rosania R, Giorgio F, Principi M, Amoruso A, Monno R, Di Leo A, Ierardi E. Effect of probiotic or prebiotic supplementation on antibiotic therapy in the small intestinal bacterial overgrowth: a comparative evaluation. Curr Clin Pharmacol. 2013 May; 8 (2):169-72.

Barone M, Della Valle N, **Rosania R**, Facciorusso A, Trotta A, Cantatore FP, Falco S, Pignatiello S, Viggiani MT, Amoruso A, De Filippis R, Di Leo A, Francavilla R. A comparison of the nutritional status between adult celiac patients on a long-term, strictly gluten-free diet and healthy subjects. *Eur J Clin Nutr*. 2016 Jan; 70 (1):23-7.

Venerito M, Helmke C, Jechorek D, Wex T, **Rosania R**, Antweiler K, Weigt J, Malfertheiner P. Leukotriene receptor expression in esophageal squamous cell cancer and non-transformed esophageal epithelium: a matched case control study. *BMC Gastroenterol*. 2016 Jul 30;16 (1):85.

Rosania R, Chiapponi C, Malfertheiner P, Venerito M. Nutrition in Patients with Gastric Cancer: An Update. *Gastrointest Tumors*. 2016 May; 2 (4):178-87.

Rosania R, Varbanova M, Wex T, Langner C, Bornschein J, Giorgio F, Ierardi E, Malfertheiner P. Regulation of apoptosis is impaired in atrophic gastritis associated with gastric cancer. BMC Gastroenterol. 2017 Jun 29; 17 (1):84.

Rosania R, Von Arnim U, Link A, Rajilic-Stojanovic M, Franck C, Canbay A, Malfertheiner P, Venerito M. *Helicobacter pylori* eradication therapy is not associated with the onset of inflammatory bowel diseases. A case-control study. J Gastrointestin Liver Dis. 2018 Jun; 27(2):119-125.

Franck C, **Rosania R**, Franke S, Haybaeck J, Canbay A, Venerito M. The BRAF Status May Predict Response to Sorafenib in Gastrointestinal Stromal Tumors Resistant to Imatinib, Sunitinib, and Regorafenib: Case Series and Review of the Literature. Digestion. 2018 Sep 4:1-6.