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Par

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# NEW ENDOMICROSCOPIC COMPUTER-BASED ALGORITHM FOR THE DIFFERENTIAL DIAGNOSIS OF INFLAMMATORY BOWEL DISEASES

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# Index

# Part 1: literature review

# Abbreviations

**AICD:** Average Inter-Cryptic Distance

**ASCA:** Anti-Saccharomyces cerevisiae antibodies

**AUC:** Area Under the curve

**CD:** Crohn Disease

**CDEIS:** Crohn Disease Endoscopic Index of Severity

**COV:** Coefficient Of Variation

**EIM:** Extra-intestinal manifestations

**FLCM:** Fluorescent Leakage through the Colonic Mucosa

**IBD:** Inflammatory bowel disease

**IEB:** Intestinal Epithelial Barrier

**MICD:** Minimal Inter-Cryptic Distance

**MVA:** Mean Vessel Area

**MVL:** Mean Vessel Length

**MVD:** Mean Vessel Diameter

**pANCA:** perinuclear AntiNeutrophil Cytoplasmic Antibodies

**PNV:** Predictive Negative Value

**PPV:** Predictive Positive Value

**ROI:** Region Of Interest

**SOW:** Size Of the Wall

**UC:** Ulcerative Colitis

# Introduction and general informations

The inflammatory bowel diseases (IBD) encompass two distinct entities: the Crohn's disease (CD) and the ulcerative colitis (UC). These pathologies are characterized by a chronic and incurable inflammation of the gastrointestinal system, alternating between recurrent activity phases of variable intensity and remissions(1,2). CD and UC are two different nosological entities: while CD can occur in any part of the digestive tract from the mouth to the anus (but mostly in the distal ileum and colon), UC expansion is limited to the rectal and colonic mucosa(3–5). Moreover the behavior of diseases varies in time and in the type of mucosa involvement as CD can cause transmural lesions such as fistulas or strictures while UC is limited to the superficial layers of the large bowel(6,7).

Extra-intestinal manifestations (EIM) can also be encountered in both diseases and represent a wide field of symptoms affecting different organs. Among those manifestations, the rheumatic ones represent the most common; a recent Chinese study involving 3153 patients with IBDs reported a rate of more than 10% of patients presenting bone or musculoskeletal manifestations of the diseases(8). The prevalence of such manifestations is even more important in western countries (10 to 62% according to studies)(9). The main others manifestations reported are ophthalmologic, coetaneous or hematologic ones(10–13).

IBD etiology remains currently unclear; the main hypothesis is considering complex, mutlifactorial diseases involving an aberrant mucosal immune response to the intestinal micro flora occurring in a predisposing genetic field and influenced by environmental factors (figure 1). A large amount of environmental parameters has been reported over time notably tobacco smoking and appendectomy, respectively protecting and aggravating factors in UC and CD, but their implication in the physiopathology of IBD are still not highlighted(14–16).

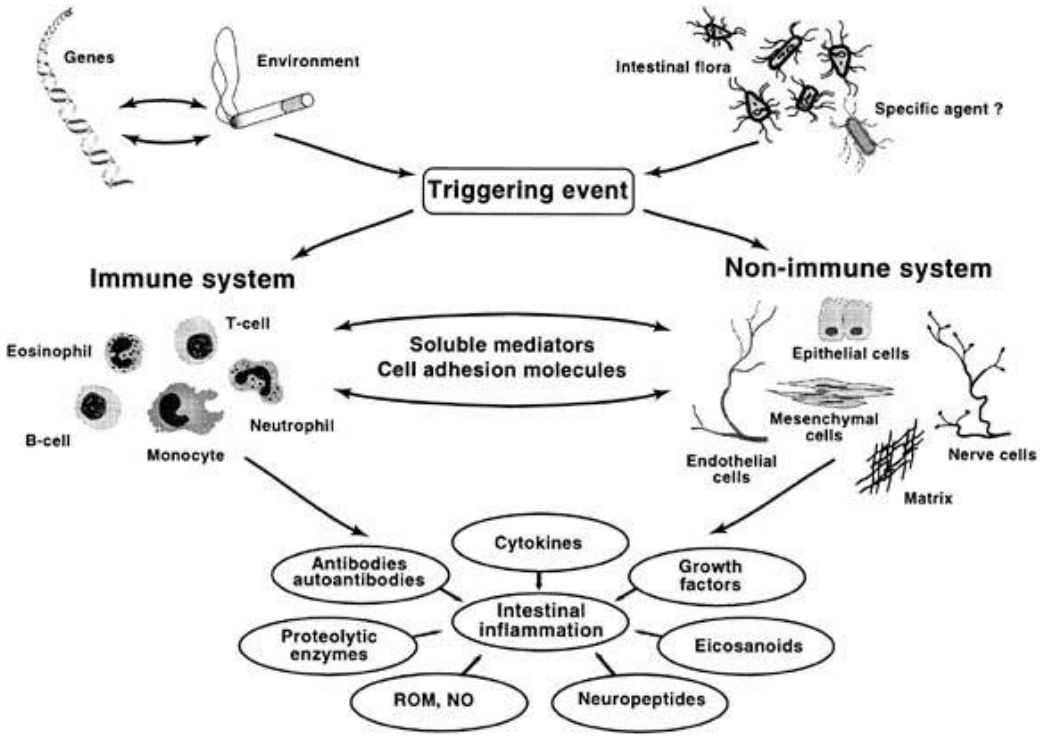


Figure 1 : diagram showing components and events involved in IBD pathogenesis according to (14)

IBD affect more than three million individual through Europe with an increasing incidence in recent years, recorded between 0.6 and 24.3 for 100000 person per year for UC and from 0.3 to 12.7 for 100000 person per year for CD according to studies(17). A North-South gradient exists in the prevalence of these pathologies with higher rates in developed countries such as northern Europe and North America but appears to raise even in developing ones(18) (figure 2).

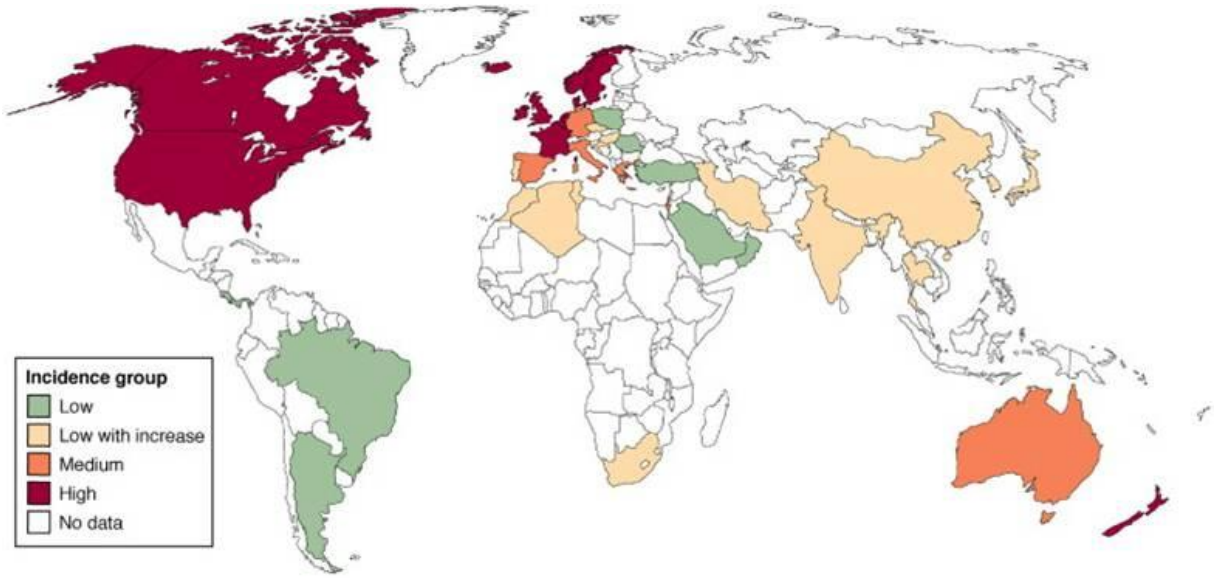


Figure 2:The global map of inflammatory bowel disease: red refers to annual incidence greater than $10/10^5$, orange to incidence of $5\text{-}10/10^5$, green to incidence less than $4/10^5$, yellow to low incidence that is continuously increasing and absence of color indicate absence of data.(18)

UC and CD mostly arise amongst young adults with a mean age at the diagnosis between 29.5 and 33.4 years old according to studies(19). Mean and median ages at diagnosis of UC are, in general, 5 to 10 years later than those associated with CD(20).Nevertheless they can be encountered at any age of life, a secondary incidence peak has being found within the fifth or sixth decades(19). Concerning the sex ratio, a slight female predominance can be observed globally, particularly in the early decades, questioning about the role of hormones on the disease appearance, but tends to reverse concerning UC(21). Ethnic differences also exist in the diseases prevalence and incidence, while African Americans have about the same risk to develop IBD; differences were shown concerning Asians or Hispanics. Nevertheless those differences could likewise be based on lifestyles as changing occurs in migrants population incidence(22,23).

The increasing incidence of these diseases and their impact on the quality of life of patient as much as health costs make them a real public health problem. Indeed, gastro-intestinal symptoms such as abdominal pain, bleeding or diarrhea may severely alter mental and physical quality of life of patient(24–28).In a recent Japanese study, less than half of the included patients were satisfied with their medical treatment and 32% had to

make adjustments such as working part-time(29). Furthermore IBD represent a public health problem in terms of health costs; a meta-analysis in 2015 reported indirect costs from $7,189.27 to $9,622.15 per year per patient; outpatient resources seems to account for the majority in the first year of diagnosis(30,31).

The diagnosis of IBD rely on a wide number of factors including clinical and endoscopic evaluation as well as histology, serology and radiology(32–34). Most of diagnosis features concern the intestinal epithelial barrier (IEB) integrity which could be a marker of the underlying inflammation as new goals of treatments tend to get to mucosal healing (35). IEB is also a component of the disease since its dysfunction, characterized by an increase in permeability, has been widely associated with IBD(36–38).

When the physician succeeds in separating IBD from functional bowel disorders, there is often a delay in diagnosis of CD and UC, sometimes more than 24 months(39). Moreover, as there are a large number of clinical presentations of IBD, it is of major importance to define the phenotype of the disease according to its severity, behavior and location in order to provide the adapted treatment(40,41). Furthermore the sub classification into either CD or UC is sometimes changing with time or impossible when the disease is only located to colon, leading to the diagnosis of indeterminate colitis (IC) or unclassified inflammatory bowel disease (UIBD).

# Indeterminate colitis

## 1- Definitions

The concept of IC was firstly introduce by Price et al. in 1978, the term referred to thirty resection specimens from which equivocal microscopic and macroscopic features couldn't allow the diagnosis of CD or UC(42). Yet this term historically defined severe acute colitis amongst cases of fulminant IBD; histological findings being consequently blurred by the severity of the inflammation which led to difficulties in the differentiation of UC and CD(43). Since then the concept of IC has been reevaluated and a new classification has been proposed by a working party in Montreal World Congress of Gastroenterology in 2005 with the appearance of the concept of UIBD for unclassified patients with clinically chronic colitis, that clearly have IBD but when definitive features of CD or UC are absent ; in resected specimens the term "colitis of uncertain type or etiology" (CUTE) should be preferred(3,44).

Nowadays the diagnosis of UIBD remains a diagnosis of exclusion leading to controversy about its definite definition. However the nosological entity of IC still remains the main diagnosis for some patients but the lack of data make it hard to apprehend(45). Meucci et al. after observing 50 patients with IC reported that the great majority (95%) of them had diarrhea at onset, 72% had bleeding diarrhea, and 74% complained of abdominal pain; a lower percentage presented with weight loss (44%) and fever (26%)(46). Furthermore, Martlan et al. tried to highlight microscopic and macroscopic peculiarities to define more specially what was to be considered histologicaly as IC (table 1) (47). In pediatric population where IC is more frequent, some authors consider it as a special form of IBD with a severe and rapid progression; Romano et al. defined five criteria for pediatric IC : abdominal pain, bleeding diarrhea, and weight loss - endoscopic macroscopic features of erosions and ulcers of the colon - pancolitis with "rectal sparing" - early onset - diffuse, transmucosal lamina propria cell increase and patchy inflammation(48).

| Macroscopic features | Microscopic features |
|---|---|
| Extensive ulceration | Extensive ulceration with a sharp transition to normal adjacent mucosa |
| Involvment of transverse and right colon (more severly than distal colon) | Transmural lymphoid inflammation, with an absence of lymphoid aggregates |
| Involvment of > 50% of the mucosal surface | Absence of well-defined, epithelioid granulomas distant from crypts |
| Usually diffuse disease, but may show rectal sparing | Multiple squat V-shaped ulcers, lacking surrounding inflammation |
| Toxic dilation may be present | Scanty deep penetrating slit-like fissure or « knife-like », spreading into the superficial half of the muscularis propria |

Table 1: morphological macroscopic and microscopic features seen in indeterminate colitis according to (47)

## 2- Epidemiology

To date only few data are available on the prevalence and incidence of UIBD over the world. It has been reported that up to 15% of new cases of IBD were stamped with the UIBD denomination(46). In Europe, the prevalence of IC has been estimated between 3 and 7 per 100 000 inhabitants (figure 3) (49). Thus it appears that the disease is significantly associated with an early onset as reported in a meta-analysis in 2009, reporting an amount of 13% of children and 6% of adults classified as UIBD (this difference was statistically significant $P < 0.0001$). However, depending on studies one should know that 50% to 80% of the patients will secondarily be reclassified between UC and CD within eight years (46,50,51). The epidemiological features of UIBD seem to be similar as for IBD concerning the sex ratio and ethnic repartition even though very few data are available concerning this issue. In a British study involving 11 432 patients with IBD, 4% had UIBD amongst whom 56% were women. The median age at diagnosis was 41 years old (11-92) showing that even if IC is more frequent in childhood the diagnosis can be made at any age (52,53).

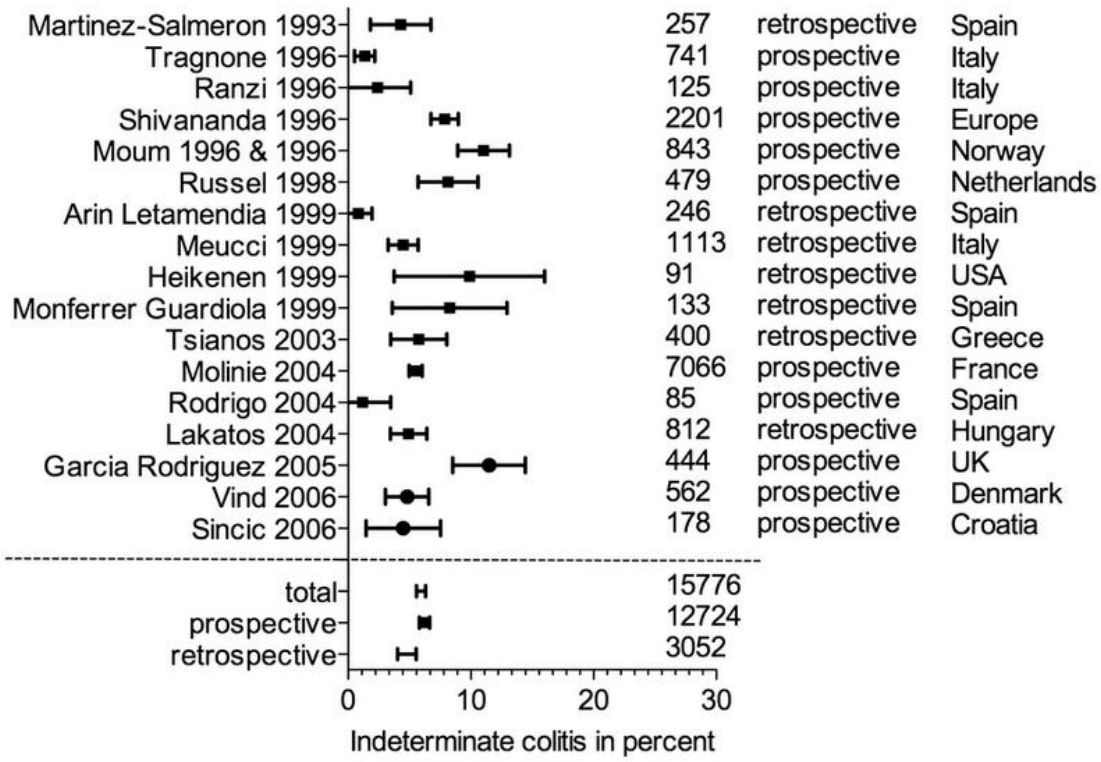


Figure 3 : indeterminate colitis prevalence in different European studies according to (54)

## 3- Consequences on care

Natural history of CD and UC implies repeated episodes of bowel inflammation leading to hospitalization, surgery and an escalation of therapy with the known effects on quality of life of patient and health costs. The goal of therapies in IBD is thus to ensure the remission of the disease. The definition of remission has changed in recent years and nowadays tends to ensure a complete mucosal healing, predictive of a sustained remission and resection-free survival (35,55–57). Thus, the medical approach in those diseases is based on drugs modulating the inflammatory response with more and more specific targets depending on the type of the disease, aiming to a personalized therapy(58–61).

Even if most drugs are currently approved for both diseases, differences in recommendations of treatment are made between UC and CD. By instance, calcineurin

inhibitors as cyclosporine remain treatment in the field of UC such as aminosalicylates that are recommended as first-line treatment in UC but do not represent as pivotal remedy in CD(40,41). Concerning the newest molecules, the American food and drug administration has recently approved the use of Golimumab for the treatment of moderate to severe UC and Natalizumab for moderate to severe CD. Differences in microbiota in those diseases also led to differences in care, fecal microbiota transplantation having showed benefices in UC while its role in CD is still unclear(62).

Another major matter concerns the surgical care in those patients. Restorative procto-colectomy with ileal-pouch-anal-anastomosis represents the reference procedure for UC patients with uncontrolled, fulminant or chronic disease(63–65). On the other hand the results are much less satisfactory for CD and UIBD. In 2005 a study involving1,270 patients revealed that pouch complications were significantly higher in patients with CD or IC (respectively 46% and 43% of patients) as compared to UC patients (22%; $P< 0.05$) (66). Another study published in 2000 reported the same type of results with more pelvic sepsis, pouch fistula or pouch failure within the UIBD group(67). This finding is all the more important as patients with IC appear to have an higher risk of colectomy(68).

## Current approach and progress in differential diagnosis

It remains unclear that IC is a separate IBD nosological entity or that diagnostic resources are still insufficient to date to separate UC from CD with atypical clinical presentations. However the criteria to define IC are currently not consensual and a high rate of misclassified patients is still do be deplored as they may receive inappropriate treatments. Therefore it is of major concern to get through this clinical issue with new biomarkers for differential diagnosis. Presently the differential diagnosis of these two entities rely on a wide, multidisciplinary beam of arguments as none gold standard has yet been developed. Thereby clinical evaluation combined with endoscopic, radiological, biological and histological features represent the current recourse (figure 4) (69).

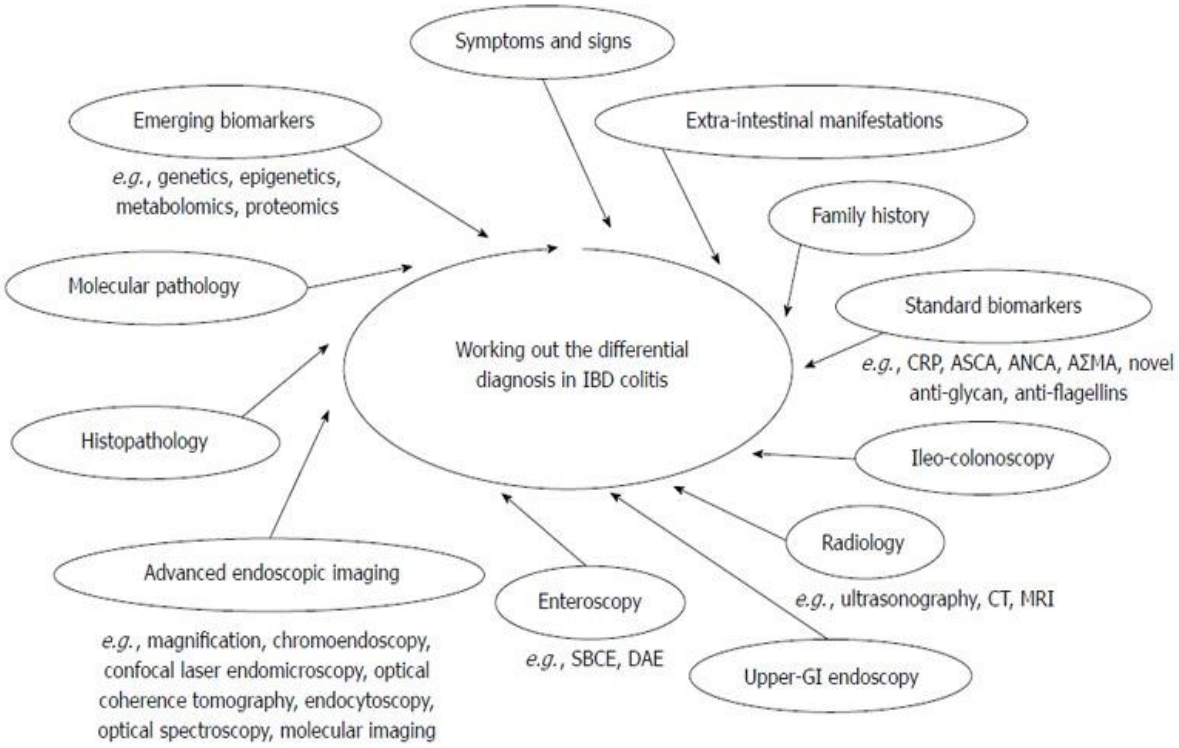


Figure 4 : current and future approach of differential diagnosis in IBDs according to (70)

## 1- Patient history and clinical manifestations

Physical findings in CD and UC may overlap considerably, however some symptoms are more likely to be found in each one. Though, different scoring systems have been developed for clinical follow-up: Crohn's Disease Activity Index (CDAI) for CD and Mayo score for UC are ones of many examples over time and point specific aspects of clinical manifestations. Thereby the main symptom of UC is visible blood in the stools reported in more than 95% of active disease. Left-sided abdominal pain, and rectal involvement related symptoms also appear as more frequent in UC(71). On the other hand, loss of weight and chronic diarrhea are more frequent in CD; Melmed et al. even showed in 2007 that non-bloody diarrhea at initial presentation ($P$ = 0.01) and weight loss >10% at presentation ($P$ = .007) were independent predictors of diagnostic change from UC to CD(72). Concerning perineal manifestations one should know that they are easily encountered through the history of CD patient, but again the distinction is not absolute

because even healthy individual may present with superficial anal fissure or uncomplicated fistulas(73,74).

As it has been mentioned above differences can also be seen concerning EIM. Dotson et al. showed as an example a statistically significant difference in the rates of EIMs between CD and UC for aphthous stomatitis, erythema nodosum, and sclerosing cholangitis in a pediatric population of 1009 patients(75). The overall rate of cutaneous manifestations seen in those diseases are also different as it has been observed by Ko et al. amongst 4147 patients in 2016(12).

Personal antecedent of smoking or appendectomy are commonly accepted as risk factors to develop either UC or CD. In a study by Bridger et al. smoking at diagnosis was associated with development of CD with an odd ratio of 3.55 ($P$ <0.001) whereas it represented a protective factor in UC (odd ratio = 0.28 $P$< 0.001)(76). In the same way, prior appendectomy is known to be associated with CD(15,77,78). Family antecedents should also be carefully considered as a hereditary tendency is frequently observed, notably in first-degree relatives. Family history thus represents the strongest risk factor of CD. Freeman et al. in 2002 reported 140 patients with parents affected with CD amongst a population of 1000 patients in a 20 years study(79). Heredity is also observed in UC but with a rate that seems inferior than in CD(80).

## 2- Biological tests

There are numerous but no specific blood tests and serological markers available for diagnosis of IBD.

Inflammation markers are indicative of extensive active intestinal inflammation and may represent a help to distinguish functional from organic disorders but not UC from CD. Various blood and stool tests exist but none is pathognomonic of one disease or the other. As example, C-reactive protein (CRP) elevated levels correspond with both diseases severity and extent but are better correlated notably with risk of surgery, active endoscopic disease or moderate-severe clinical activity in CD (81,82). On the other hand CRP has been shown to correlate with clinical activity and disease extent in UC(83).

Anti-*Saccharomyces cerevisiae* antibodies (ASCA) and perinuclear Anti-Neutrophil Cytoplasmic Antibodies (pANCA) are widely used for the differential diagnosis since they were the first developed in this indication. A meta-analysis involving 3,841 UC patients and 4,019 CD patients published by Reese et al. in 2006 reported a sensitivity of 54.6% and a specificity of 92.8% for the diagnosis of CD with the $ASCA^+$ and $pANCA^-$ test result(84). Furthermore, it appears that ASCA and pANCA may predict not only the occurrence of IBD years before the diagnosis, but also characteristics of the diseases.CD patients with $pANCA^+$ have mostly a UC like presentation with colonic involvement whereas increased titers of ASCA are correlated with young age at onset, ileal involvement and structuring or penetrating behavior or perineal involvement (85–88).

New biomarkers have been developed over time, thus antibody to *Escherichia coli* outer membrane porin, bacterial flagellin and *Pseudomonas fluorescens* have been tested in this indication but it has been proven that they were not predictive of a change in diagnosis(72). Anti-chitobioside carbohydrate antibodies, anti-laminaribioside carbohydrate antibodies, anti-mannobioside carbohydrate antibodies, anti-chitin IgA and anti-laminarin IgA have been found positive in at least one third of CD patients negative for ASCA, but their overall sensitivity in CD was low(Table 3) (87,89).Concerning UIBD, this type of tests is particularly relevant. A study led by Joossens have enrolled from 1996 to 2002 97 patients with UIBD, 32% were reclassified in either CD or UC; in these patients $ASCA^+/pANCA^-$ correlated with CD in 8 of 10 patients, whereas $ASCA^-/pANCA^+$ correlated with UC in 7 of 11 patients, only 7 seronegative cases (14.9%) became CD or UC compared with 48% (24 of 50) of seropositive patients ($P < 0.001$) (90).

| Marker | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | AUC |
|---|---|---|---|---|---|
| **ASCA** | 39–66.1 | 85–95 | 54–95.4 | 38.2–68.4 | 0.76–0.85 |
| **ACCA** | 8.7–20.7 | 84.9–97 | 78–87.1 | 24.2–52 | 0.53–0.69 |
| **ALCA** | 15–25.6 | 92.3–96 | 78–90.3 | 25–53 | 0.63–0.81 |
| **AMCA** | 12–27.3 | 82.4–97 | 65–91.7 | 25.5–52 | 0.59–0.66 |
| **Anti-C** | 10–25.6 | 92.7–98 | 88–91.1 | 30–39 | 0.65 |
| **Anti-L** | 18–25 | 89.5–97 | 87.4–90 | 29.1–41 | 0.7–0.71 |

Table 3: Antiglycan antibodies in differentiation of CD vs. UC (ACCA, anti-chitobioside carbohydrate IgA antibodies; ALCA, anti-laminaribioside carbohydrate IgG antibodies; AMCA, anti-mannobioside carbohydrate IgG antibodies; Anti-C, anti-chitin carbohydrate antibody; Anti-L, anti-laminarin carbohydrate antibody) according to (89).

## 3- Endoscopy

Endoscopy plays a central role in the management of IBD; it represents a macroscopic diagnostic tool with well-defined scoring systems, allows the realization of targeted specimens with histological aim and represents a therapeutic recourse in many complication cases. The typical endoscopic findings in patient with either CD or UC have been profusely described in literature over time(91,92). Yet, typical cases of UC usually present with edematous mucosa, erythema, loss of vascular markings, and mucosal friability with an ascendant lesions extend proximally in a continuous, confluent and concentric fashion with clear demarcation of inflammation. More severe cases may be associated with erosions, ulcers, and spontaneous bleeding (figure 5) (93).

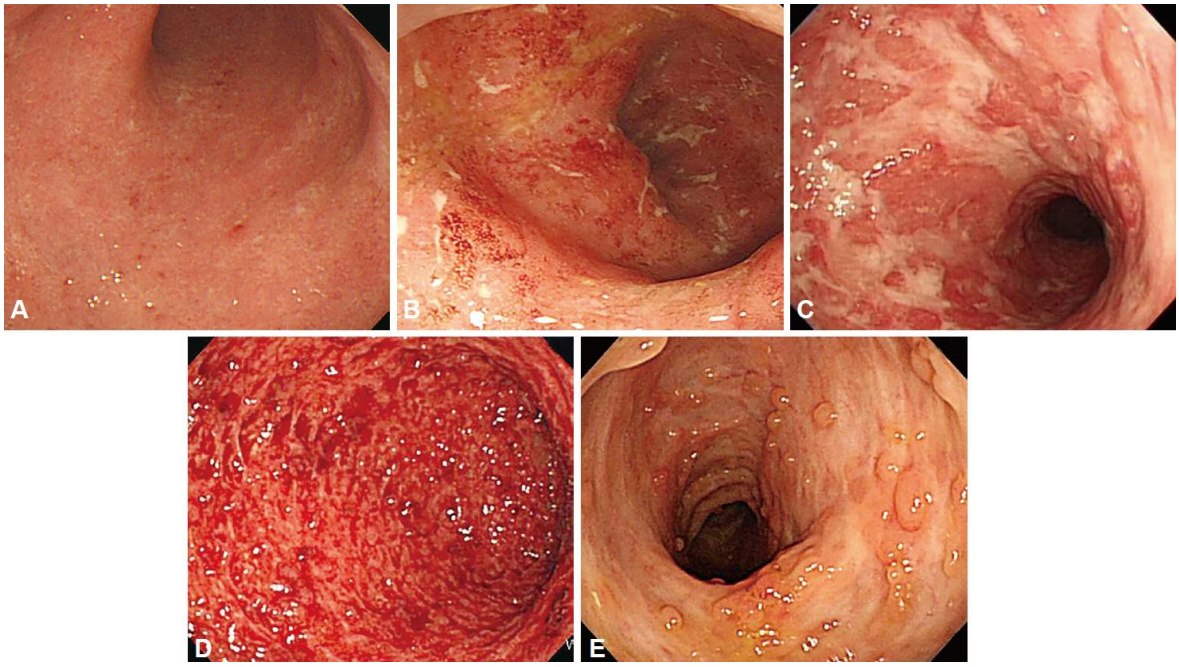


Figure 5: Typical endoscopic features of ulcerative colitis. (A) Mild: mucosal erythema, fine granularity, decreased vascular marking. (B) Moderate: marked erythema, loss of vascular marking, erosions. (C) Severe: ulcers. (D) Severe: spontaneous bleeding. (E) Luminal narrowing with pseudo polyps according to (93)

Unlike ulcerative colitis, Crohn's disease may occur within any area of the digestive tract showing asymmetrical, discontinuous, focal, and patch characteristics with longitudinal ulcers, cobblestone appearance or small aphthous ulcerations arranged in a longitudinal fashion mucosa surrounding the ulcers usually appearing normal or almost normal. Useful endoscopic features can also be seen in complicated diseases with fistulas or strictures (figure 6).

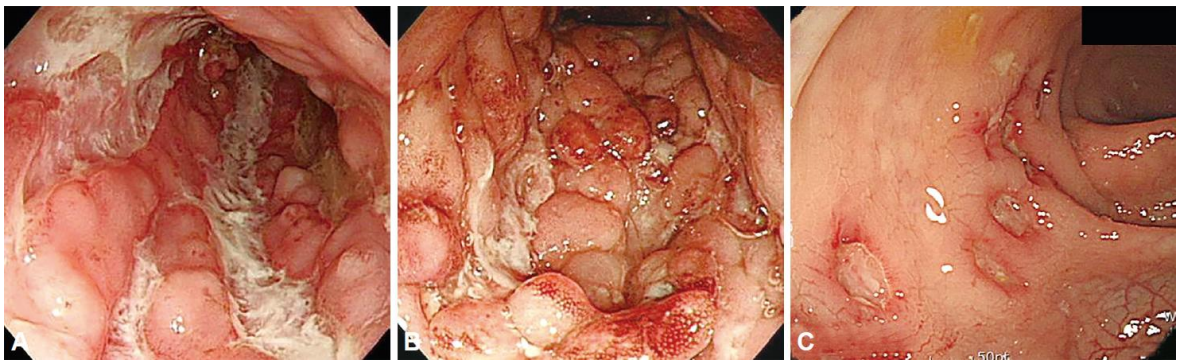


Figure 6: Typical endoscopic features of Crohn's disease. (A) Longitudinal ulcers,(B) cobblestone appearance, (C) aphthous ulcers showing longitudinal array according to (93).

In a prospective study conducted within 357 patients, Petra et al. found an accuracy of colonoscopy of 89%, with 4% errors and 7% indeterminate diagnoses, highlighting the endoscopic features mentioned above as the most relevant (94). However, no endoscopic feature is considered to be specific for CD or UC (91). Thereby other pitfalls exist in the differentiation of IBD; firstly, inflammation often appears segmental with relative rectal sparing and patchiness after the first lines of treatment (95). Otherwise, unusual inflammation locations can be observed in UC notably in the upper intestinal tract as it has been observed in several studies (96,97). Another matter of fact is the concept of backwash ileitis which typically occurs in up to 20% patients with pancolitis and can mimic an ileal involvement, confusing the practitioner (98). Small bowel assessment holds thereby just as much an important place in the differential diagnosis. Wireless Capsule Endoscopy (WCE, figure 7)and enteroscopy represent two of the pathways through its evaluation(99). Even if WCE present with good specificity and sensibility (respectively 77% and 89%), its main advantage lies in its negative predictive value (96%)taking into account other causes of damage to the small intestine such as non-steroidal anti-inflammatory drugs and is of great help in patients reclassification (100,101). Enteroscopy, whether assisted by balloons or with an overtube, presents less convincing results of detection of the lesions but makes it possible to carry out biopsies or therapeutic intervention(99).

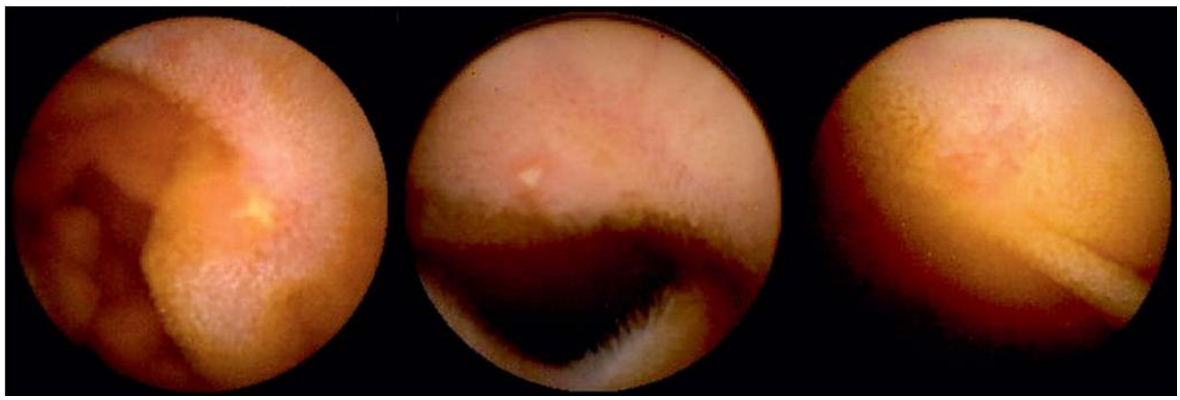

Figure 7 : Lesions as seen at WCE (aphtoids ulcers) according to (99)

Innovations in endoscopy have been developed lately, related to technologic advances allowing a better mucosal characterization. Thus, high definition, magnification and dye-less chromoscopy represent an advance in the potential of endoscopy concerning lesions detection but no evidence has been brought yet that they could help in differentiating UC from CD(70). Going deeper in the analysis of mucosa, confocal laser endomicroscopy (CLE) and endocytoscopy allow *in vivo* microscopic analyses and may play a role in this indication. Tontini et al. even developed an endomicroscopic diagnostic scoring system with an accuracy of 94% (102). Multiphoton microscopy has still a superior resolution and does not require exogenous fluorophores but has only be tested ex vivo in IBD patients (103). Finally, optical coherence tomography (OCT) was tested by Shen et al. among 70 patients with IBD; the disrupted layered structure on OCT was indicative of transmural inflammation and had a diagnostic sensitivity and specificity of 90.0% and 83.3% for CD, respectively (104). The future in endoscopy might be represented by molecular in vivo imaging which seems promising as a topical fluorescein labeled DAS-1/CG-3 antibodies application and detection described by Yantiss et al (105).

## 4- Histopathology

The histological examination of endoscopic biopsies or resection specimens remains a key step in the work-up of affected patients and represents nowadays the gold standard in differential diagnosis. Recommendations of expert are to perform a minimum of two biopsies from at least five sites along the colon, including the rectum, and the terminal ileum. The biopsies should be collected in separate vials and fixed immediately in formaldehyde-based fixative(106). The usual histological findings in IBD are related in table 4.

| | Ulcerative colitis | Crohn's disease |
|---|---|---|
| **Crypt architectural irregularity** | Diffuse (continuous) | Focal (discontinuous) |
| **Chronic inflammation** | Diffuse(continuous)<br>Decrease proximally | Focal (discontinuous)<br>Variable |
| **Patchiness** | Uncommon | Common |
| **Localization** | Superficial<br>Transmucosal | Transmural |
| **Serositis** | Absent except in fulminant colitis | Present |
| **Lymphoid aggregates** | Frequent in mucosa, submucosa | Common, transmural |
| **Granulomas** | Absent, except with ruptured crypts | Present |
| **Acute inflammation** | Diffuse (continuous) | Focal (discontinuous) |
| **Crypt epithelial polymorphs** | Diffuse (continuous) | Focal (discontinuous) |
| **Crypt abscesses** | Common | Uncommon |
| **Mucin depletion** | Present, pronounced | Uncommon, mild |
| **Neuronal hyperplasia** | Rare | Common |
| **Muscular hypertrophy** | Absent | Present |
| **Paneth cell metaplasia** | Present | Uncommon |
| **Pyloric gland metaplasia** | Rare | Present |

Table 4 : Microscopic features used for the diagnosis of IBD according to (106)

However, despite the well-established criteria summarized here that allow an accuracy of 66-75 %, many pitfalls exist in the histological diagnostic of UC or CD leading to less accurate diagnosis (107,108). Even in the presence of specific features, expert pathologists may not be consensual; Theodossi et al. reported a range of inter-observer agreement between 65 and 76% in this issue(109). As in endoscopy, unusual locations of inflammation as caecal patch or through backwash ileitis or even related with a discontinuous distribution with rectal sparring secondary to treatment can be confusing(106).

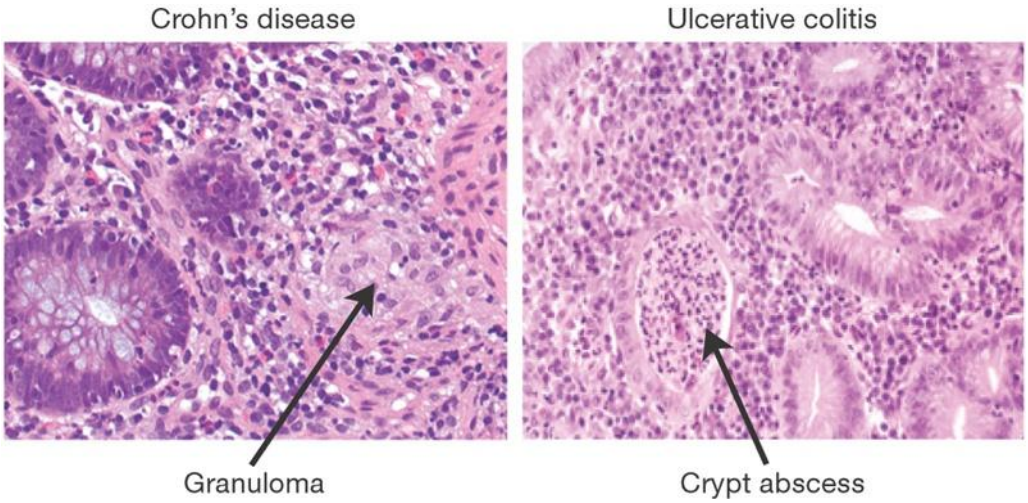


Figure 8 : illustration of histological findings in CD (left panel) and UC (right panel) according to (110)

In a contemporary review in 2015 causes leading to uncertainty in IBD pathology were listed by Odze, reporting that most causes of uncertainty are due to overlapping features as a result of fulminant colitis, insufficient clinical, radiologic, endoscopic or pre-resection biopsy information, or failure to recognize unusual pathologic variants of UC that mimic CD, and variants of CD that mimic UC (111).

Here again prospects are opening up for the future with new molecular techniques to refine accuracy and decrease uncertainty. Yantiss et al. reported alterations in the immune-histochemical expression of Das-1 and CG-3 in colonic mucosal biopsy as a help to distinguish IBD; suppression of Das-1 staining occurs more frequently in UC (96%) compared with CD (20%) ($P< 0.001$) while CG-3 positivity in crypt epithelium was significantly more common in UC (52%) ($P \leq 0.02$)(105).

## 5- Radiology

In the last decades, multiple imaging technologies have been developed that improve visualization of the mucosal, mural and perienteric inflammation associated with inflammatory bowel disease(32). Thus, cross-sectional imaging techniques are an important adjunct to endoscopic assessment, to allow a complete and sensitive staging of the small bowel and perineum with the unique advantage to assess mural and extramural

disease(112).Furthermore, their use in patients with IBD permits detection of clinically occult inflammation and penetrating and extra-enteric complications. The choice of the radiological investigation strongly depends on the local expertise and availability among centers and advantages and disadvantages balance.

- Ultra-sonography (US) is non-invasive and does not impart ionizing radiation while being widely available. It has shown good results in CD detection with a sensitivity and specificity of 88% and 93% respectively with a bowel wall thickness threshold greater than 3 mm, but is dependent on local expertise and only permit a limited visualization of gastro-intestinal tract (113).
- Computed Tomography Enterography (CTE) present the advantage of assessing the entire abdomen and pelvis with a spatial resolution. It is also available in most centers and is recommended as complementary to endoscopy, detecting clinically occult disease but expose patient to the risk of radiation and iodine complications.
- Magnetic Resonance Imaging (MRI) allows for an accurate assessment of the small bowel without radiation exposure, making this imaging tool ideally suited to the CD population given their age and need for repeated imaging. The sensitivity and specificity of MRI for the diagnosis of CD was reported by Panes et al. at 78% and 85% respectively(114).

A meta-analysis comparing the accuracies of US, MRI, scintigraphy, CT, and Positron emitted tomography for diagnosis in patients with suspected or known IBD, showed that mean sensitivity estimates for the diagnosis of IBD were high and not significantly different among the imaging modalities (90%, 93%, 88%, and 84% for US, MRI, WBC scintigraphy, and CT, respectively)(115).

Solutions for the future may also reside in the combination of imaging and endoscopy, the spearhead of such techniques being represented by echo-endoscopy. In a recent study this technique allowed the differential diagnosis of IBD based on thickening of the mucosa in UC ad of the sub-mucosal layers in CD more or less the presence of para-colonic nods(116).

## 6- Genetic

Over the last ten years a wealth of susceptibility loci have been discovered, enhancing the comprehension of UC and CD genomic profiles. In 2013 a meta-analysis based on 15 genome wide association studies including more than 75000 cases and controls identified 71 new associations, for a total of 163 IBD loci that met genome-wide significance thresholds. These data have confirmed the existence of an important overlap in genetic risk factors and that most loci contribute to both IBD phenotypes (117).

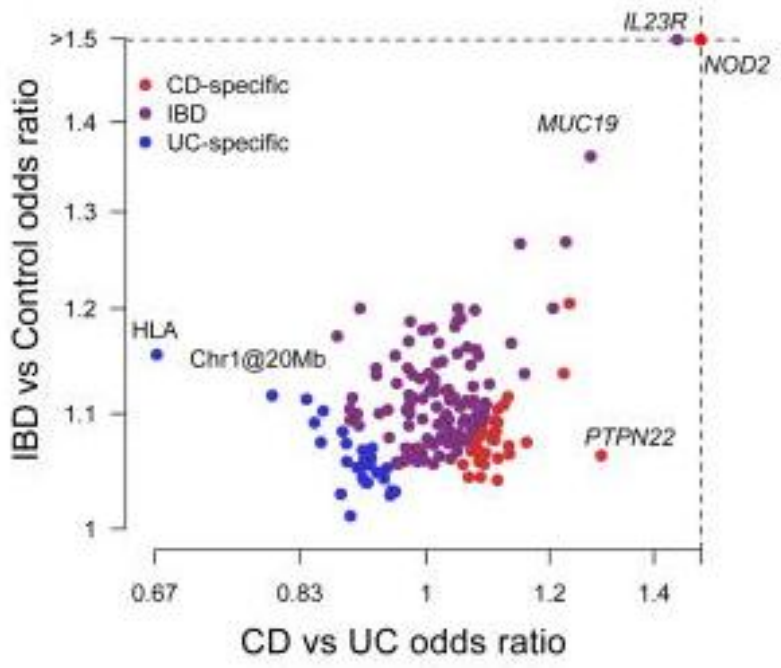


Figure 9 :Graphic representing the 193 independent signals, plotted by total IBD odds ratio and phenotype specificity (measured by the odds ratio of CD relative to UC), and colored by their IBD phenotype classification according to (117). Notice the NOD2 and IL23R position related to their discrimination capabilities.

Among the genetic links in IBD, the most representative may be NOD2/CARD15 and IL23R gene variants that have been found to play an important role in the susceptibility to Crohn's disease(118).A multigene analysis led by Von Stein et al. in 2008 identified seven marker genes allowing the differential diagnosis between IBD with area under the receiver-operating characteristic curves ranging from 0.915 to 0.999 ($P < .0001$), showing the increasing interest of genetic findings in this indication(119).

Gene-environment interactions affecting gene expression with no changes in the DNA sequence, called epigenetics, and mainly represented by cytosine-guanine dinucleotides methylation, is another matter of concern in IBD pathogenesis and expression. Even though no evidence of epigenetics findings have yet been reported, their potential in IBD diagnosis is already pressed(120).

# Endomicroscopy

## 1- Generalities – history

Confocal endomicroscopy is a recently developed tool from the spectrum of endoscopy, allowing in vivo microscopic evaluation of the digestive mucosa during a conventional endoscopic evaluation. This technique is based on the use of a single optic fiber applied directly on the mucosa, beforehand impregnated with fluorescent substance (either locally applied with acriflavin hydrochloride or intravenously with fluorescein) and lighting the region of interest by laser excitation with a wave length of 488 nanometers. Images produced this way are then numerically treated to obtain only those from the desired focal plan, allowing more or less “optical biopsies” with a x1000 magnification, an observation field diameter of 475 micrometers and a lateral resolution of 7 micrometers (figure 9). The optical fiber can be either contained in a probe introduced through the operating channel (probe-based confocal laser endomicroscopy (pCLE); Cellvizio, Mauna Kea Technologies, Paris, France) or directly integrated in the distal end of the endoscope (integrated confocal laser endomicroscopy; Pentax Europe GmbH, Life care)(121,122).

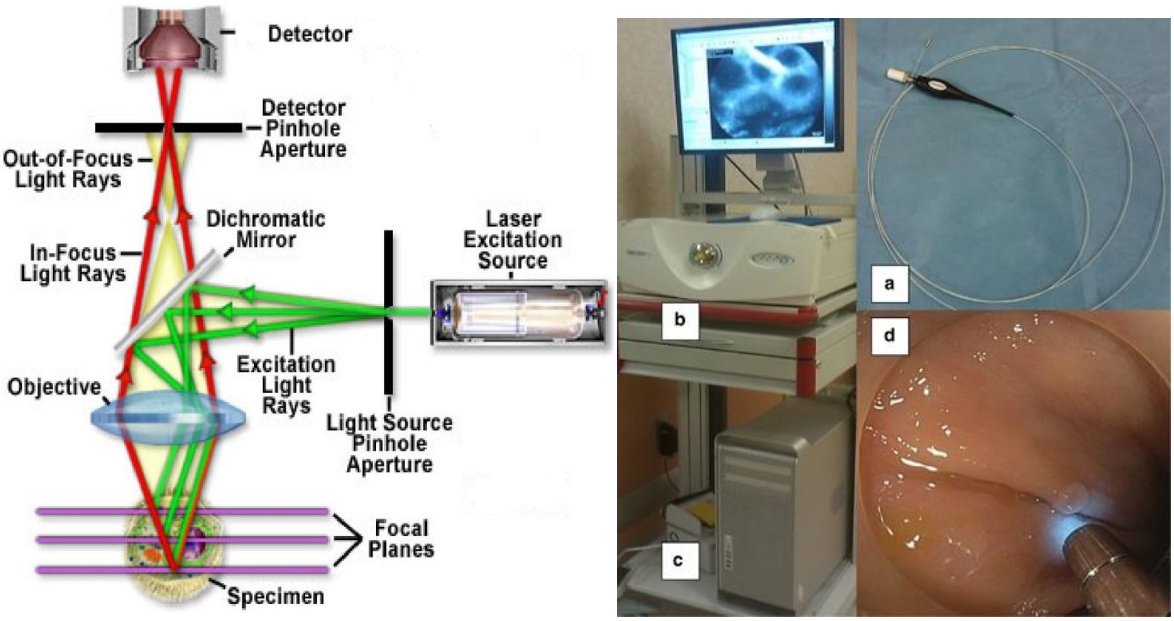


Figure 9: principles of endomicroscopy: on the left, diagram representing the optical principles of endomicroscopy, the laser excitation source lighten the region of interest (i.e. the colonic mucosa) previously impregnated with fluorescent substance at a wave length of 488 nm via a set of mirrors and lentils, fluorescence is then detected through a pinhole aperture and numerically treated. On the right : Picture representing a) endomicroscopic probe, b) Laser scanning unit, c) Computer processing unit and d) Endomicroscopic probe applied on the colonic mucosa during recording.

Even if Kiesslich et al. have firstly orientated the use of endomicroscopy in usual practice toward colonic neoplasia screening, numerous new indications and uses have been developed over time. Thereby detection of dysplasia in Barrett's esophagus, gastric cancer evaluation, assessment of pancreatico-biliary strictures or prediction of response to treatment have been evaluated with strongly interesting results (121). Other studies also focused on the characterization of mucosal inflammation (123). Lim et al. notably showed that a dynamic analysis of the Intestinal Epithelial Barrier (IEB) was possible and permitted do highlight lesions that neither histopathology nor conventional endoscopy could detect (124). A correlation between histology and endomicroscopy has been beside proved by Zambelli et al. in a collagenous colitis model or by Musquer et al. for CD patients with modifications in the architecture of the crypts (125,126). Finally in relation to our actual issue, endomicroscopy has also been widely developed in various indications for IBD management.

## 2- Endomicroscopy in IBD

The use of endomicroscopy in IBD management has recently been extrapolated to numerous objectives(127). Neumann et al. have attempted to define a CD activity score based only on endomicroscopic; thus, the colonic crypt tortuosity, an enlarged crypt lumen, micro erosions, augmented vascularization, and increased cellular infiltrates within the lamina propria were associated with more active disease(128). In the same way, Watanabe et al. demonstrated that the colonic crypts of active ulcerative colitis showed large, irregular arrangement with numerous inflammatory cells, dilated capillaries were visible on the lamina propria and an increased fluorescein leakage through colonic mucosa(129).

Hundorfean et al. and Tontini et al. as far as they are concerned tried to differentially diagnose CD from UC with semi-quantitative criteria counting for surface irregularity and architectural distortion (figure10).

The prediction of a relapse of IBD was also studied by Kiesslich et al. via the analysis of dysfunction of the intestinal epithelial barrier by highlighting an active leakage of fluorescein through the surface epithelium as a sensitive criterion. Liu et al. proposed a quantitative measure called epithelial gap density, defined as a total number of epithelial gaps counted on pCLE images, as a means to identify early mucosal barrier dysfunction and inflammation; increasing gap density was found to be predictive of aggressive disease and subsequent clinical relapses in IBD patients.

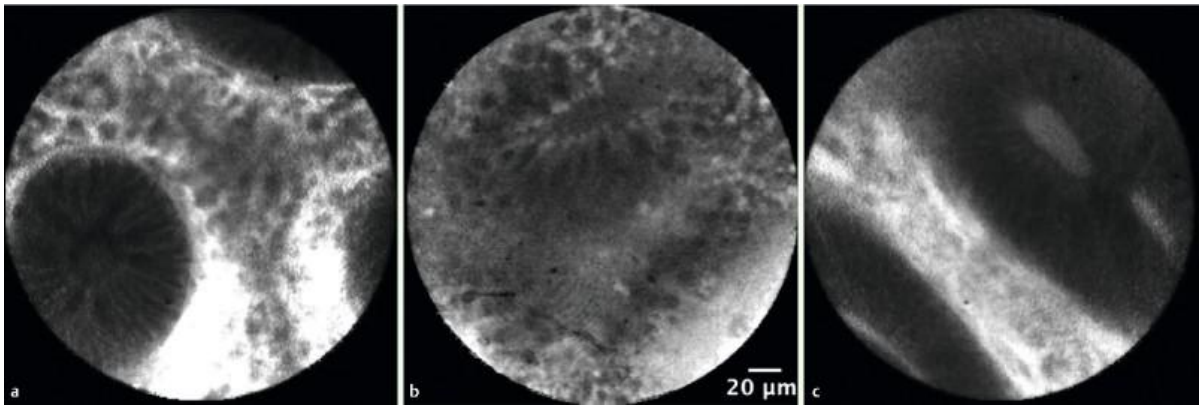


Figure 10 : Confocal laser endomicroscopy findings : a) Normal colonic crypts in a healthy individual, b) Focal cryptitis in a patient with active Crohn's colitis, c) Crypt architectural distortion in a patient with active ulcerative colitis according to (102).

Screening for colorectal cancer in IBD patient has also been a matter of concern as expert recommendations propose a high number of randomly spotted biopsies all along the colon as the binding reference method. In line with its first purpose, endomicroscopy has thus been evaluated for its capacity to detect dysplasia among those patients; a meta-analysis in 2013 reported a sensitivity of 83 % and a specificity of 90 % in this indication(130).

## 3- Issues and limitations

Even if endomicroscopy appears as an interesting and promising tool, it certainly presents few limitations. Firstly, the technique is currently in the clinical research stage, being used only among expert centers. There are still no recommendations of use in any diagnostic algorithm and it hasn't found its place yet in the clinical common practice. Its use, in any case in France, is still not reimbursed by the social security and raises evenly the question of costs.

Furthermore, and in the direct consequences of these findings, there are still no consensual criteria in any indications. Even if classification have been developed or some features, as the fluorescence leakage or the surface irregularity inherited directly from histopathology, are found in many studies about mucosal inflammation, the way of apprehending those remains deeply heterogeneous among literature(131).

Finally, despite its computer-based technology, endomicroscopy is still depending on the evaluator's expertise and background(132).Moreover, the technique can become rapidly time-consuming as the better results are obtained by offline watched recordings, according more precise and focused analysis(133).All this without counting the necessary training time needed to learn such a new diagnosis method. Chang et al. reported in 2011 a learning curve certainly reasonable but still necessary before acquiring sufficient efficiency and accuracy(134).

A place thus exists for the development of quantitative and objective consensual criteria, probably simplified by computer-based analysis as pilot studies have already proposed(135).

## Conclusion and perspectives

CI and UIBD still remain subject to controversy in many points as their precise definition, care and even existence is not consensual. However, differentiating CD from UC appears to be an important issue as many patients in this case may receive inappropriate therapy and undergo repeated diagnostic tests, bringing this issue to the attention of public health considerations.

Even if progress have been made in the last decade, the differential diagnosis between CD and UC, particularly in their colonic or fulminant form, currently based on a cluster of multidisciplinary arguments as a pathognomonic test is still to find. Perspectives for the future may be found in molecular pathology, genetic findings or endomicroscopy but still need to be improved before they can be extrapolated to a common practice.

Thereby, we propose here a new approach in the use of endomicroscopy, with computer-based quantitative findings with the goal to overpass the expertise needed for this tool and help its democratization.

# Part 2 – Original article

# Introduction

The diagnosis of inflammatory bowel diseases (IBD) rely on a wide number of factors including clinical and endoscopic evaluation as well as histology, serology and radiology (32–34). The histological evaluation includes the investigation of acute and chronic inflammatory cell infiltrates, crypt abscesses, mucin depletion, surface epithelial integrity, and crypt architectural irregularities(136,137). Most of features concern the intestinal epithelial barrier (IEB) integrity which could be a marker of the underlying inflammation. IEB is also a component of the disease since its dysfunction, characterized by an increase in permeability, has been associated with IBD (36–38). IEB assessment is thus central to diagnose IBD.

In 2004, confocal laser endomicroscopy (CLE), an endoscopic technique for microscopic assessment of the gastrointestinal mucosa, was introduced into clinical practice (138). CLE was initially used to predict the colonic neoplasia, but further studies aimed to assess mucosal inflammation in the gastrointestinal tract. CLE has been widely used in patients with IBD such as Crohn's disease (CD) and ulcerative colitis (UC). These studies revealed that CLE can be used to detect *in vivo* the histological changes associated with IBD (131). Subsequently several score were proposed to predict relapse and inflammation activity of IBD (139,140). Surprisingly very few studies have assessed the role of CLE in initial diagnosis of IBD (102,129,141).

When the physician succeeds in separating IBD from functional bowel disorders, there is often a delay in diagnosis of CD and UC, sometimes more than 24 months (39). Moreover the sub classification into either CD or UC is impossible for around 10% of patients which are labeled as having an indeterminate colitis (142) (IC). Making this differential diagnosis is essential as optimal treatment and management of both diseases is different (102). We have studied mucosa from patients in clinical remission with the aims to obtain representative situation of IC patients for which the first flare was controlled. As mucosal healing is an increasing goal to IBD (143), patients in remission could allowed to assess it. Mucosal healing is used to lead the decision of dose escalation, or the switching or stopping of biological therapies (144). Furthermore it has been

showed that histological inflammation persists even when endoscopic mucosal healing is reached. Recent data tend thereby to demonstrate that histological remission could be a new goal to step aside from disease relapse (145).

To date few studies have performed quantitative analysis of mucosal changes with CLE technique, while the feasibility has already been demonstrated (125,140,146). This appears to be a significant point especially as developing quantitative analysis through objective and measurable parameters would permit to bypass the learning curve and the expertise needed for CLE practice.

We hypothesized that computer-based measurements may reveal details of the structure and the function of colonic mucosa of IBD patients in clinical remission related to the former inflammation. We found that measured parameters could be potential biomarkers of IBD and could be used to differentiate CD from UC.

# Materiel and methods

## Design

Consecutive adult patients who were diagnosed with CD or UC or cancer screening between 2009 and 2016 and underwent CLE at the Gastroenterology Department of the University Hospital Nantes (France) and the European Hospital Georges Pompidou (Paris, France) were retrospectively screened. Selected CD patients were in clinical remission, defined by a Harvey Bradshaw Index ≤ 5 at the time of enrollment, and in endoscopic remission defined by a Crohn's disease endoscopic index of severity (CDEIS) ≤ 7. Selected UC patients were also in clinical and endoscopic remission, defined by Mayo score ≤ 1 at the time of enrollment. Patients with no personal or family history of CD and UC who were undergoing colonoscopy for screening or surveillance of polyps/cancer served as control group. Demographic data (gender, age) and IBD characteristics (age at diagnostic, IBD extension based on Montreal classification, disease duration) were recorded at inclusion. Patient could be secondly excluded from the study if the endomicroscopic recording quality was insufficient *i.e*. 1) if the total number of analyzable crypts after mosaicking was less than 35, 2) if the recording was performed in the 10 firsts minutes or after 20 minutes after fluorescein injection, 3) if the duration of the recording was less than five minutes or 4) if clinical data were missing.

## Confocal laser endomicroscopy

All the endomicroscopic videos were recorded during a colonoscopy with the probe based system Cellvizio© and the Coloflex© probe (Mauna Kea Technologies, Paris, France) introduced through the endoscope operating channel and connected to a laser scanning unit and a data processing unit for recording. Recording was processed by endoscopist within 20 minutes after a 10% intravenous fluorescein sodium injection of 5 to 15 ml according to the patient size and weight and the fluorescence intensity on screen. Recorded movies duration had to be at least of 5 minutes. The colonic areas recorded were left to the endoscopist discretion as patients were in endoscopic

remission. All bowel preparations were performed using polyethylene glycol. Recordings were conducted by five endoscopists among whom three were not endomicroscopy experts.

We have characterized the mucosa with fourteen parameters:

- 13 architectural parameters (Figure 1):
    - The perimeter.
    - The sphericity, defined by$4 \times \pi \times \text{Area} / \text{Perimeter}^2$, expressed as a percentage.
    - The roundness defined by the normalized ratio between the radii of the minimum and maximum circles written in the form, expressed as a percentage.
    - The maximal Feret diameter named Feret and defined by the maximal distance between 2 points of the perimeter.
    - The elongation factor named EF and defined by the ratio between the minor diameter and the major diameter.
    - The Ma/ma ratio named Ma/ma and defined by ratio between the width and the height of the box containing the crypt.
    - The density defined by the ratio of the crypt area and the area of the field of view
    - The mean vessel area, named MVA and defined by the ratio of the vessels area and the area of the field of view
    - The mean vessel length (MVL)
    - The mean vessel diameter (MVD).
    - The minimal (MICD) and the average (AICD) distance between the geometrical centers of neighbor crypts.
    - The Wall thickness named WT and defined by the distance between nearest neighbor crypts.

- 1 functional parameter named Fluorescein leakage through the colonic mucosa (FLCM) and defined by the increase of fluorescence of mucosa trough the time.

In order to account for the surface irregularity, which appears to be a relevant criterion of mucosal inflammation, we evaluated the coefficient of variation (COV, *i.e.* the relative measure of data dispersion around the mean, in percentage) of each parameter(106). We assessed that a significant variation of the values could be assimilated to a greater surface irregularity.

## Software, programs and calculations

As recording artefacts due to patient and endoscopist movements hinder a detailed analysis, recorded videotapes were firstly investigate with the Cellvizio Viewer © software (Mauna Kea Technologies, Paris, France) to perform mosaicking, which consisted in a 2D reconstruction of the probe scanned surface. This operation resulted in the creation of "mosaics" illustrated in figure 2.

Once the mosaic was obtained, intrinsic architectural parameters of the crypts were calculated using the Icy software(147). To perform the calculation we used the Active Cells plug-in that implements an active contour segmentation method using exponential splines as basics functions to represent the outline of the crypt, computing a region of interest (ROI). Corresponding geometric data were thus obtained via the ROI statistics tool *i.e.* the perimeter, the sphericity, the roundness, the Feret, the EF and the Ma/ma.

Concerning the crypt density measurement, we have developed a macro in ImageJ(148) software to calculate the ratio between the field of view area and the sum of area crypt. The measurement of MICD, AICD and WT have been adapted from a plug-in developed by Haeri et al.(149).

The vessel parameters were measured using the IC Viewer version 3.8.6© Vessel Detection© plug-in. This plug-in enabled an automatic detection of the vessels directly from each endomicroscopic records frames, based on fluorescence intensity detection with a threshold set manually at 10 µm. We reported the vessels length and area to the field of view area to normalize data.

Finally the FLCM was measured using the "signal analysis" tool of the Cellvizio Viewer© software. As the Cellvizio© confocal endomicroscopic probe in clinical version

proceeds internal calibration we could not compare directly fluorescence intensity between patients. Thus, we calculated the FLCM as the increase of fluorescence intensity reported to time unit, independent from calibration.

## Statistical analysis

Statistical analysis was performed using the Graphpad Prism software (Graphpad Prism 5.0, Graphpad Software Inc.).Mean comparisons were performed using non parametric Mann Whitney test. Area under the curve of receiver operating characteristic (AUROC), multivariate regression and the logistic model were performed using STATA 14 (Stata Corp LP).

Differences with p-value off 0, 05 or less are considered as statistically significant. The following symbols transcribe significance: * = p < 0, 05; ** = p < 0, 01; *** = p < 0,001; **** = p < 0, 0001.

# Results

## Patients

We have analyzed 409 movies from 69 colonoscopies obtained from 70patients who underwent colonoscopy at the Gastroenterology department of University hospital of Nantes between 2009 and 2016. 3 patients were excluded despite corresponding to the inclusion criteria because of insufficient video quality or crypt number after mosaicking, 8 patients were excluded because of missing data (Figure 3).35 patients (60%) were man and the mean age was 50 years old. The mean age of CD patients was 40 years old which was significantly younger (p = 0.0063). CD extension was including the upper gastrointestinal tract in 1 (4%) patient, terminal ileum in 1 (4%) patient, ileo-colic in 18 (78%) patients and colic in 3 (13%) patients. Disease phenotype was mostly non-structuring, non-penetrating (57%). UC extension was proctitis in 6 (22%) patients, left side colitis in 16 (60%) patients, and pancolitis in 5 (18%) patients. Disease duration was respectively of 15 years for CD and 16 years for UC. Baseline demographic characteristics are available in Table 1. No adverse event in connection with fluorescein injection was noted. No difference was noted concerning the recordings quality between endoscopists accustomed to endomicroscopy and novices.

## Mucosal cryptometry of controls and IBD patients with no clinical sign of inflammation

The control group was constituted of 9 patients; a total surface of 5985 $mm^2$ was analyzed (*i.e.* a mean of 665 $mm^2$ per patient) corresponding to 866 crypts (*i.e.* a mean of 96 crypts per control) have been measured to establish the average crypt dimensions (*i.e.* cryptometry) in physiological condition (Table 2 – Mean column). The IBD group was constituted of 50 patients, a total surface of 78970 $mm^2$ was analyzed (*i.e.* a mean of 1574 $mm^2$ per patient) corresponding to 5399 crypts (*i.e.* a mean of 107 crypts per

patient) have been measured to set the average cryptometry in inflammatory condition (Table 2).

Interestingly, the cryptometry of mucosa of IBD in remission significantly differed from control mucosa. Increase in the perimeter (594.1± 211.4 vs. 748.1 ± 163.5, p=0.0159, control vs. IBD) and in the Feret diameter (194.9 ± 80.0 vs. 273.3 ± 65.9, p=0.0027, control vs. IBD) reflected larger crypts in IBD mucosa. Increase in the AICD (316.7± 93.6 vs. 492.5 ± 70.1, p=0.0000, control vs. IBD) and in the WT (170.4± 66.1 vs. 321.5 ±65.37, p=0.0000, control vs. IBD) revealed largest gap between crypt and a perturbation of crypt distribution. Increase in FLCM (7.17± 4.62 vs. 21.27 ± 14.50, p=0.0057, control vs. IBD) eventually unveiled a significant increase of mucosal permeability.

As illustrated in figure 4A and presented in table 2 (COV column), not only the mean value of parameters change in IBD mucosa but also the relative measure of data dispersion around the mean (*i.e.* coefficient of variation, COV, expressed in percentage).Comparison of control to IBD group has shown that except for the sphericity and the density of crypt, all COV was greater in the IBD group (perimeter, p=0.0021; roundness, p=0.0057; Feret, p=0.0001; EF, p=0.0020; Ma/ma, p=0.0036; MVL, p=0.0114; MVA, p=0.0330, MVD, p=0.0114, AICD, p=0.0000; MICD, p=0.0001; WT, p=0.0006).

## IBD diagnosis from cryptometry analysis

Area under the curve of receiver operating characteristic (AUROC) was performed for each parameter and COV to evaluate their power of discrimination (Table 3). The COV of the maximal Feret diameter ($COV_{Feret}$) is the most powerful character to separation control and IBD group with an AUROC of 98%. We next applied a multivariate logistic regression of $COV_{Feret}$ on the other parameter (Table 3). The addition of $COV_{Feret}$ to the intercrypt distance or to the wall thickness (WT) increased the AUROC to 100%. Thus, only the measurement of 2 parameters was needed to fully separate control and IBD group.

We have next calculated a score allowing us to diagnose IBD from our computer-assisted measurements. This score (named IBDiag) was calculated as followed:

$$\textbf{IBDiag} = -361.498+13.42*\boldsymbol{COV_{Feret}} +0.590*\boldsymbol{WT}$$

In our population, IBDiag was negative in control group while IBDiag was always positive in IBD group (Spe and Se = 100%, Figure 4B).

## Mucosal cryptometry of CD and UC patients with no clinical sign of inflammation

The CD group was constituted of 23 patients, a total surface of 34627mm² was analyzed (*i.e.* a mean of 1505 mm² per patient) corresponding to 2340 crypts (*i.e.* a mean of 108 crypts per patient). The UC group was constituted of 27 patients, a total surface of 44345 mm² was analyzed (*i.e.* a mean of 1642 mm² per patient) corresponding to 3059 crypts (*i.e.* a mean of 113 crypts per patient). Both groups have been measured to set the average cryptometry in two pathological conditions (Table 2).

Despite remission of disease in both groups, several differences were significantly observed such as the EF which is smaller in the UC group than in the CD group (1.45 ±0.18 vs. 1.36±0.16, p=0.0493). Concerning the distribution of the crypt in the mucosa, we have shown that the AICD (468.3± 78.4 vs. 513.0 ± 55.6, p=0.0211), the MICD (264.8 ± 37.2 vs. 293.9 ± 32.5, p=0.0079) and the WT (294.4± 69.5 vs. 344.6 ± 52.6, p=0.0032) were increased in the UC group as compared to CD group. Concerning the vascularization, we have observed a decrease in the MVL (0.018± 0.003 vs. 0.016 ± 0.003, p=0.0275) in the UC group as compared to the CD group. We eventually measured an increase in the fluorescein leakage (15.2± 8.3 vs. 26.5 ± 16.7, p=0.0051) in the UC group as compared to the CD group.

The COV measurement revealed a larger distribution of density values (34.6± 8.9 vs. 47.6 ± 25.2, p=0.0048) and a smaller distribution of WT values (45.8± 6.5 vs. 41.6 ± 6.7, p=0.0070) in the UC group as compared to the CD group (Table 2).

## Differential diagnosis of CD and UC

As previously, AUROC was performed for each parameter then follow with a multivariate logistic regression of significant parameters described in the previous paragraph (Table 4). This analysis allowed us to compute a score (named IBDif) as followed (figure 4C):

$$\mathbf{IBDif} = -24.520+0.123*\mathbf{FLCM}-0.400+\boldsymbol{COV_{WT}}+0.138*\boldsymbol{COV_{MVA}}$$
$$+0.194*\boldsymbol{COV_{Perimeter}}+0.066*\mathbf{MICD}+653.943*\boldsymbol{MVL}$$

Using the IBDif score, we defined a calculation to predict the probability to properly diagnose UC rather than CD as followed:

$$\mathbf{P_{UC}} = e^{IBDif} / (1-e^{IBDif})$$

IBDif differentially diagnosed our IBD population with a Sensitivity of 92.3% and a specificity of 91.3% (figure 7). The probability cut off between UC and CD is illustrated in figure4C. The area of diagnostic uncertainty corresponded to a probability in the interval [0.5-0.75] to successfully diagnose UC rather than CD. The probability of making the right diagnosis grew then exponentially with either the sensitivity for UC or the specificity for CD. Using IBDif, only two patients of each group would have been misclassified (Figure 4D).

# Discussion

In the present study, we have demonstrated that computer-based measurements of CLE images can be used for the differential diagnosis of IBD. We quantitatively assessed the endomicroscopic morphology of the crypts pit pattern (i.e. the newly described cryptometry), vessels and fluorescence increase into colonic mucosa and used it to propose a process leading to the identification of the IBD subtype. Our findings suggest that computer-based analysis have a high potential for *in vivo* imaging in human with a broad application in endoscopy.

Among the different morphologic criteria that could be used to characterize colonic mucosa, the pit pattern has already been validated in high magnified endoscopy to detect changes during precancerous stages (150). The pit opening axes have also been correlated with stages of CD and the histological analysis(125). Despite difference in method of analysis or in technology, we noticed that in these two studies a mean of Ma/ma of 1.6 was characteristic of normal colonic tissue, a value close to that obtained in our study.

Although both CD and UC are marked by an excessive mucosal immune response and share many symptoms, they are two distinct physipathological entities(151). The differential diagnosis of the two diseases is of vital importance for the optimization of clinical management, as therapies and reliable prognostic indices often involve disease-specific strategies(32,33,102). In addition, a specific diagnosis is particularly relevant for surgical therapy. Restorative procto-colectomy with ileo-pouch anal anastomosis is the main surgical treatment for UC patients(152), while colorectal CD frequently requires an ostomy at an early branching point(153). However the reclassification of patient's diagnosis after surgery commonly occurs in 4-9% of the patients(154,155).

Tontini et al. have recently proposed the IDEA score to differentially diagnose CD and UC(102). This score was calculated from observation of the CLE images by the operator. These authors eventually oriented diagnosis towards UC instead of CD with a sensitivity of 97.4% and a specificity of 90.0%. Here, we have obtained a sensitivity of 92.3% and a specificity of 91.3%, confirming the high potential of endomicroscopy in this indication. While the specificity of the two procedures is quite similar, the sensitivity in our study is

weaker; that may be explained by the fact that in his study Tontini included consecutive IBD patients with and without endoscopic signs of inflammation. In ours, only IBD patients in clinical and endoscopic remission were included. This selection would represent the situation for UIBD which is classified later after the fare period as we know treatments can make it more difficult for pathologists to assess the diagnosis of either UC or CD(111). The IDEA score was established by expert operators while we used computerized measurements without expert point of view. Moreover, Chang et al. showed that the learning curve for CLE was of main importance as a difference in diagnosis accuracy of about 13% existed between experienced and inexperienced analysts(156). It should surely be of great interest to compare human and computer analysis.

In our study, we developed a new scoring system based on architectural and functional quantitative parameters: the IBDif score. We used the following criteria as they appear to be the most relevant for the differential diagnosis: the wall thickness, the fluorescence leakage through colonic mucosa, the minimal inter-cryptic distance and the mean vessel length, combined with the COV of the mean vessel area and of the perimeter. The use of COV to evaluate the different parameters can be perceived as a way to mathematically account for the surface irregularity as reported for histological or endomicroscopic diagnostic criteria(102,106). The fact that in almost every parameter the COV was higher in IBD than in control patients shows that this way of quantitatively apprehending the surface irregularity works, and that the architectural distortion remains a major parameter in the microscopic evaluation of the IBD.

The density, defined as the crypt number per unit area of mucosa, has been highlighted in UC to be correlated with the severity of the inflammation(140). We have defined the density as the area of crypt per area of mucosa in order to integrate crypts partially visible on the edges of the image. Our measure of density could so be affected not only by the number of crypt but also by the size of crypts. This same study used the fluorescein density of CLE images to quantify fluorescein leakages in crypt lumen. However, we didn't use the same CLE system. We have collected data with the Cellvizio (Mauna Kea Technologies) instead of the Pentax system. The Cellvizio, in this clinical version, uses internal calibration procedures, that didn't allow comparing fluorescence intensity between on/off cycles. Thus we calculated the FLCM as the increase of

fluorescence intensity per time unit. This value was independent of tools calibration and has allowed us to compare objectively patients.

Biases probably exist in this new method as quantitative endomicroscopic findings have yet very little been studied; we are, to our knowledge, the first to have used so many quantitative criteria for endomicroscopic assessments. As an example, the pressure with which the probe is applied during the endoscopy can distend the colonic mucosa and artificially increase the inter-cryptic distances. Other pitfalls may reside in the intra or inter-individual differences. Variations of the sizes and density of crypts could vary, depending on the patients' body mass index or the colonic location as it has never been studied. Furthermore, with this new method, only the analysis phase is computer-based and there are still needs for stable images recorded during the endoscopic phase to allow a sufficient quality. Nevertheless we didn't find any difference in the quality of recordings in either endomicroscopic experts or beginners.

We have also tried to highlight correlations between the parameters previously described and clinical, biological or endoscopic findings. Yet, we couldn't show the existence of such links with neither the sex, the age of the patients and the disease duration, neither with the appearance of an advert event such as surgery or a disease outbreak. Similarly we haven't found any correlations concerning CRP levels, the histologicaly assessed levels of inflammation or endoscopic severity scores (CDEIS and Mayo score). The absence of correlation found here may probably be explained by the fact only patients in remission were included, thereby reducing greatly the differences that might exist.

The main limitation of this study is eventually its retrospective nature. Besides, as the procedure has been developed from already classified patients, the importance of identified markers in this study is still not clear in the diagnosis of IC patients. Further validation of the computer-based differential diagnosis will be necessary in a prospective trial. Thus, the application of this diagnostic procedure would be easily implemented into clinical practice, as colonoscopies are routinely performed in IBD patients to assess the extent and severity of mucosal inflammation(143).

## Tables and figures

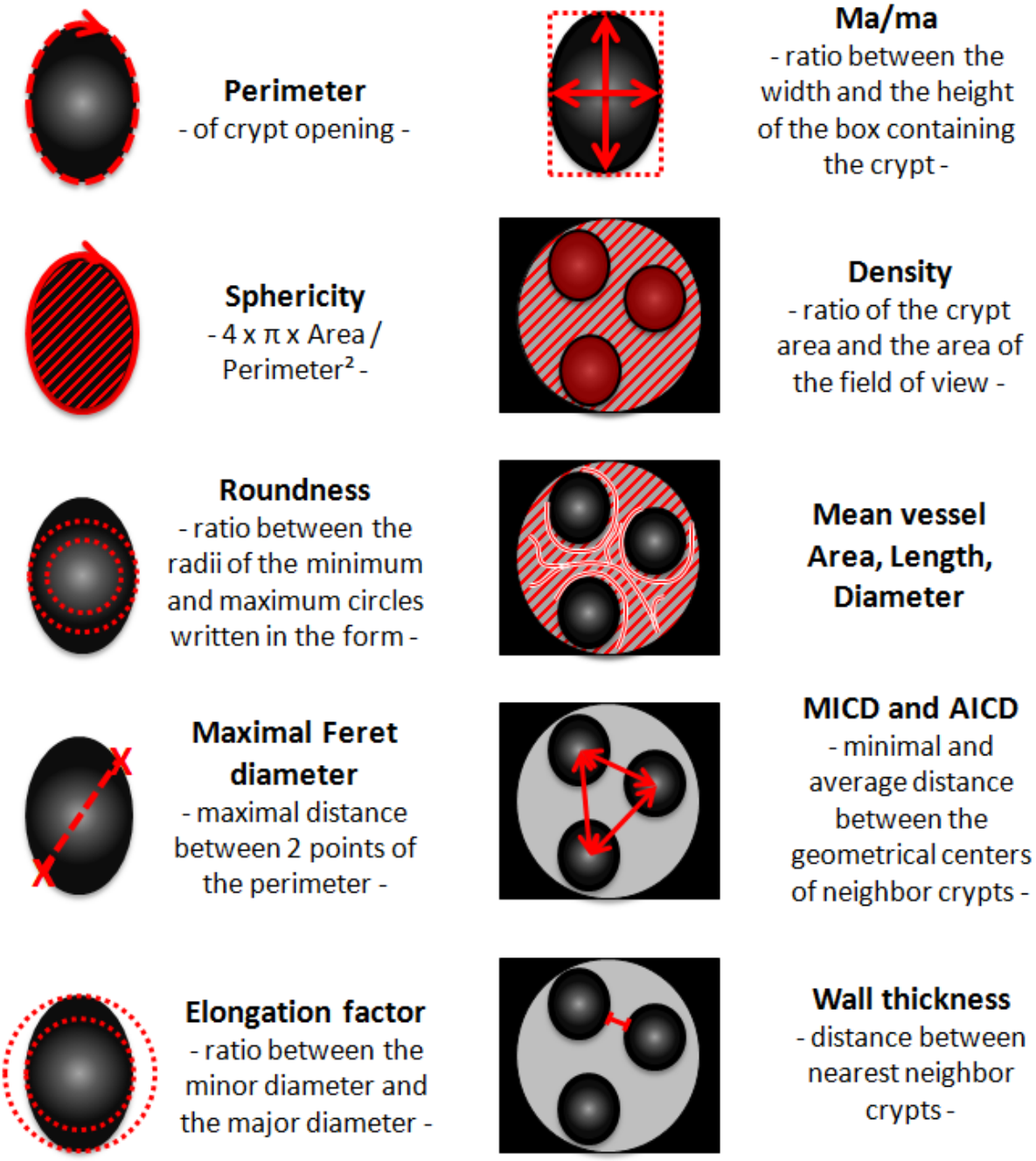


Figure 1: Illustration of the architectural parameters used for the crypts characterization.

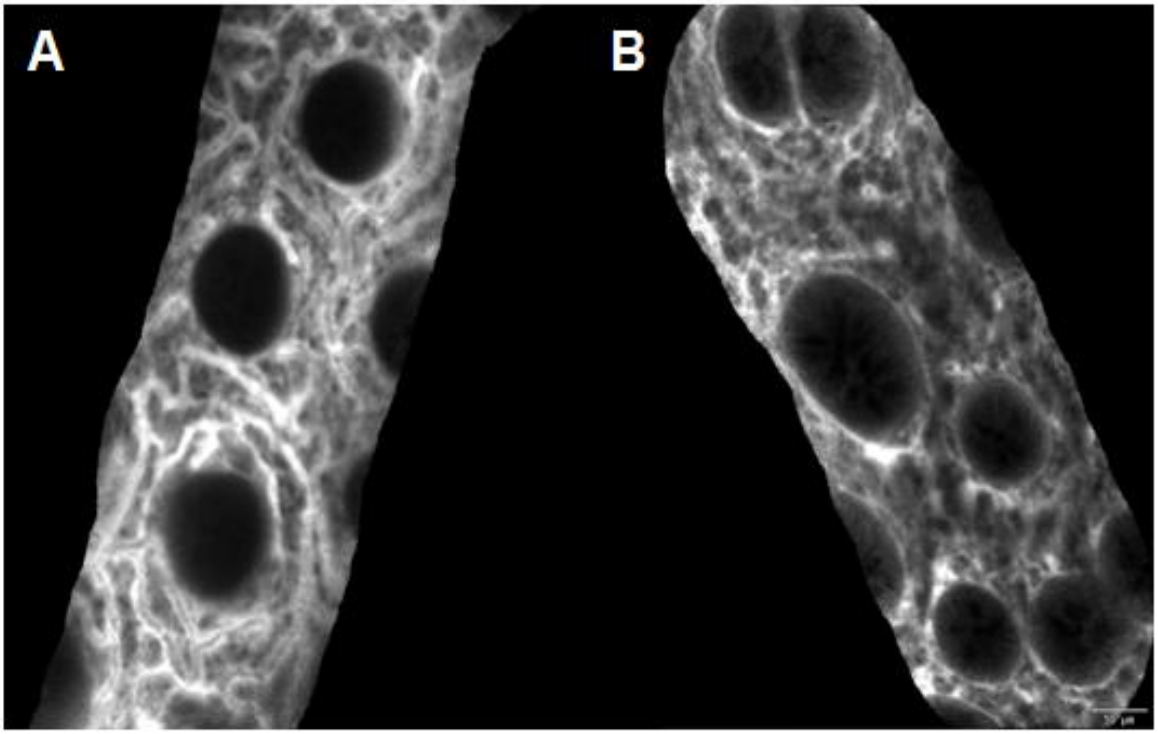


Figure 2: Endomicroscopic findings after mosaicing process; A) healthy patient mucosa with a regular distribution of spherical crypts of the same size and B) UC patient's mucosa in remission with an irregular repartition of the crypts, appearing less spherical shapes and with different sizes, one can notice a crypt fusion or scission on the top of the picture.

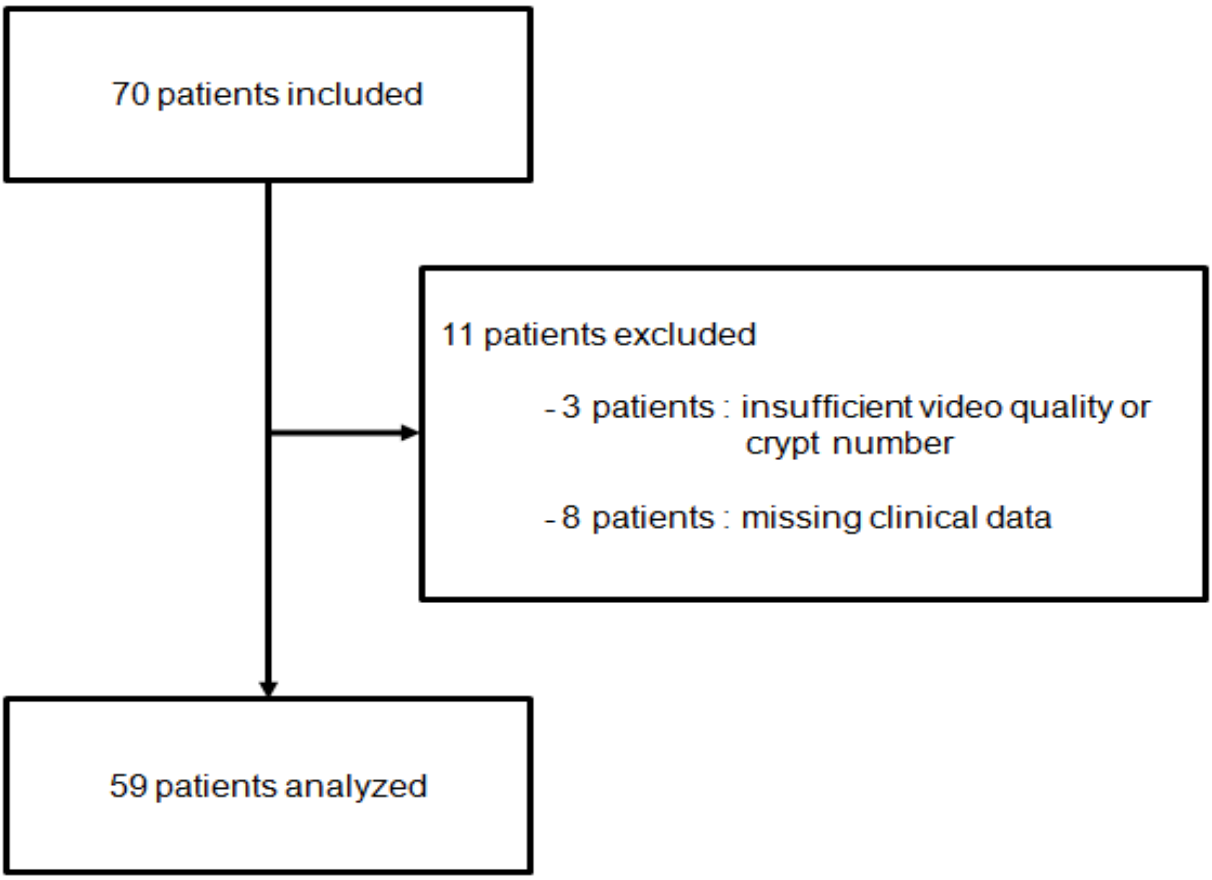


Figure 3: Flow chart.

| | Controls | CD | UC |
|---|---|---|---|
| n | 9 | 23 | 27 |
| Age, mean (min-max),years | 58 (29-79) | 40 (18-74)[a] | 50 (22-71) |
| Sex, n (M/F) | 7/2 | 13/10 | 15/12 |
| Disease duration, median, years | NA | 15 | 16 |
| CD phenotype, n (%) ( Classification) | | | |
| Non stricturing, non-penetrating | NA | 13 (57) | NA |
| Stricturing | NA | 7 (30) | NA |
| Penetrating | NA | 3 (13) | NA |
| CD location, n(%) | | | |
| Terminal ileum | NA | 1 (4) | NA |
| Colon | NA | 3 (13) | NA |
| Ileo-colic | NA | 18 (78) | NA |
| + Upper GI | NA | 1 (4) | NA |
| Perineal[(1)] | NA | 7 (30) | NA |
| CD clinical activity (Harvey Bradshaw Index), median (95 % IC) | NA | 0 (0-1) | NA |
| CD endoscopic activity (CDEIS), median (95 % IC) | NA | 2 (0-6) | NA |
| UC location, n(%) | | | |
| Proctitis | NA | NA | 6 (22) |
| Left colitis | NA | NA | 14 (52) |
| Extensive colitis | NA | NA | 2 (8) |
| Pancolitis | NA | NA | 5 (18) |
| UC clinical activity (CAI), median (95% IC) | NA | NA | 0 (0,11-0,53) |
| UC endoscopic activity (Mayo score), median (95% IC) | NA | NA | 0,5 (0-1) |
| Antecedent of surgery [(2)] | NA | 5 (22) | 0 (0) |
| Treatment received at the time of endoscopy, n(%) | | | |
| Salazopyrin | NA | 7 (30) | 13 (48) |
| Budesonide | NA | 2 (9) | 0 (0) |
| Methotrexate | NA | 1 (4) | 0 (0) |
| Imurel | NA | 6 (26) | 4 (14) |
| Anti TNF | NA | 5 (22) | 8 (30) |
| Other[(3)] | NA | 2 (9) | 2 (8) |

[(1)] None of the patients had only perineal or upper GI location

[(2)] Ileo-caecal resection in 4/5 cases

[(3)] 2 patients didn't have treatment, 2 patients received Ciclosporin or Tacrolimus

[a] ($p = 0,0063$) one-way ANOVA with Dunn's post-test.

Table 1: Patients demographic characteristics at baseline.

| Parameter | Control | | IBD | | Crohn's disease | | Ulcerative colitis | |
|---|---|---|---|---|---|---|---|---|
| | Mean(±S.D.) | COV(±S.D.) | Mean(±S.D.) | COV(±S.D.) | Mean(±S.D.) | COV(±S.D.) | Mean(±S.D.) | COV(±S.D.) |
| Perimeter (µm) | 594.1 (± 211.4) | 13.66 (± 4.39) | 748.1 (± 163.5) | 28.60 (± 13.69) | 765.2 (± 137.1) | 26,3 (± 10.3) | 733.5 (± 163.5) | 30.5 (± 16.0) |
| Sphericity (%) | 97.29 (± 2.40) | 3.34 (± 4.09) | 95.08 (± 3.52) | 5.39 (± 4.29) | 95.0 (± 3.4) | 5,8 (± 4.1) | 95.1 (± 3.7) | 5.1 (± 4.5) |
| Roundness (%) | 73.83 (± 11.88) | 13.24 (± 2.96) | 68.10 (± 12.20) | 22.77 (± 9.81) | 66.5 (± 11.8) | 23,5 (± 8.8) | 69.4 (± 12.6) | 22.2 (± 10.7) |
| Max Feret diameter (µm) | 194.9 (± 80.0) | 13.43 (± 2.25) | 273.3 (± 65.9) | 29.26 (± 11.41) | 281.0 (± 73.0) | 27,9 (± 9.0) | 265.0 (± 59.6) | 30.4 (± 13.2) |
| Elongation Factor | 1.27 (± 0.14) | 13.10 (± 2.84) | 1.40 (± 0.18) | 23.12 (± 9.16) | 1.45 (± 0.18) | 24,7 (± 8.9) | 1.36 (± 0.16) | 21.8 (± 9.3) |
| Major axis / minor axis | 1.204 (± 0.116) | 11.84 (± 4.10) | 1.254 (± 0.090) | 18.82 (± 6.65) | 1.28 (± 0.08) | 20,1 (± 6.1) | 1.23 (± 0.09) | 17.7 (± 7.0) |
| Density | 0.238 (± 0.052) | 27.80 (± 15.39) | 0.314 (± 0.120) | 41.60 (± 20.41) | 0.335 (± 0.100) | 34,6 (± 8.9) | 0.296 (± 0.133) | 47.6 (± 25.2) |
| MVL | 0.018 (± 0.003) | 17.94 (± 7.04) | 0.017 (± 0.003) | 41.55 (± 36.89) | 0.018 (± 0.003) | 33,4 (± 9.6) | 0.016 (± 0.003) | 39.7 (± 19.9) |
| MVA | 0.233 (± 0.041) | 19.13 (± 7.24) | 0.213 (± 0.039) | 44.09 (± 52.14) | 0.233 (± 0.041) | 34,9 (± 10.8) | 0.203 (± 0.039) | 34.5 (± 11.1) |
| MVD (µm) | 13.01 (± 0.88) | 8.69 (± 1.97) | 12.47 (± 0.72) | 11.46 (± 2.29) | 12.6 (± 0.8) | 12,0 (± 2.7) | 12.4 (± 0.7) | 11.0 (± 1.8) |
| AICD µm) | 316.7 (± 93.6) | 17.29 (± 3.63) | 492.5 (± 70.1) | 27.60 (± 4.64) | 468.3 (± 78.4) | 27,8 (± 4.1) | 513.0 (± 55.6) | 27.4 (± 5.1) |
| MICD (µm) | 253.1 (± 66.2) | 17.31 (± 4.11) | 280.5 (± 37.4) | 25.27 (± 5.22) | 264.8 (± 37.2) | 24,6 (± 3.7) | 293.9 (± 32.5) | 25.8 (± 6.3) |
| Wall thickness (µm) | 170.4 (± 66.1) | 34.19 (± 8.33) | 321.5 (± 65.37) | 43.54 (± 6.85) | 294.4 (± 69.5) | 45,8 (± 6.5) | 344.6 (± 52.6) | 41.6 (± 6.7) |
| FLCM | 7.17 (± 4.62) | N.A. | 21.27 (± 14.50) | N.A. | 15.2 (± 8.3) | N.A. | 26.5 (± 16.7) | N.A. |

Table 2: Cryptometry findings among control, IBD (UC and CD patients results combined), UC and CD patients.

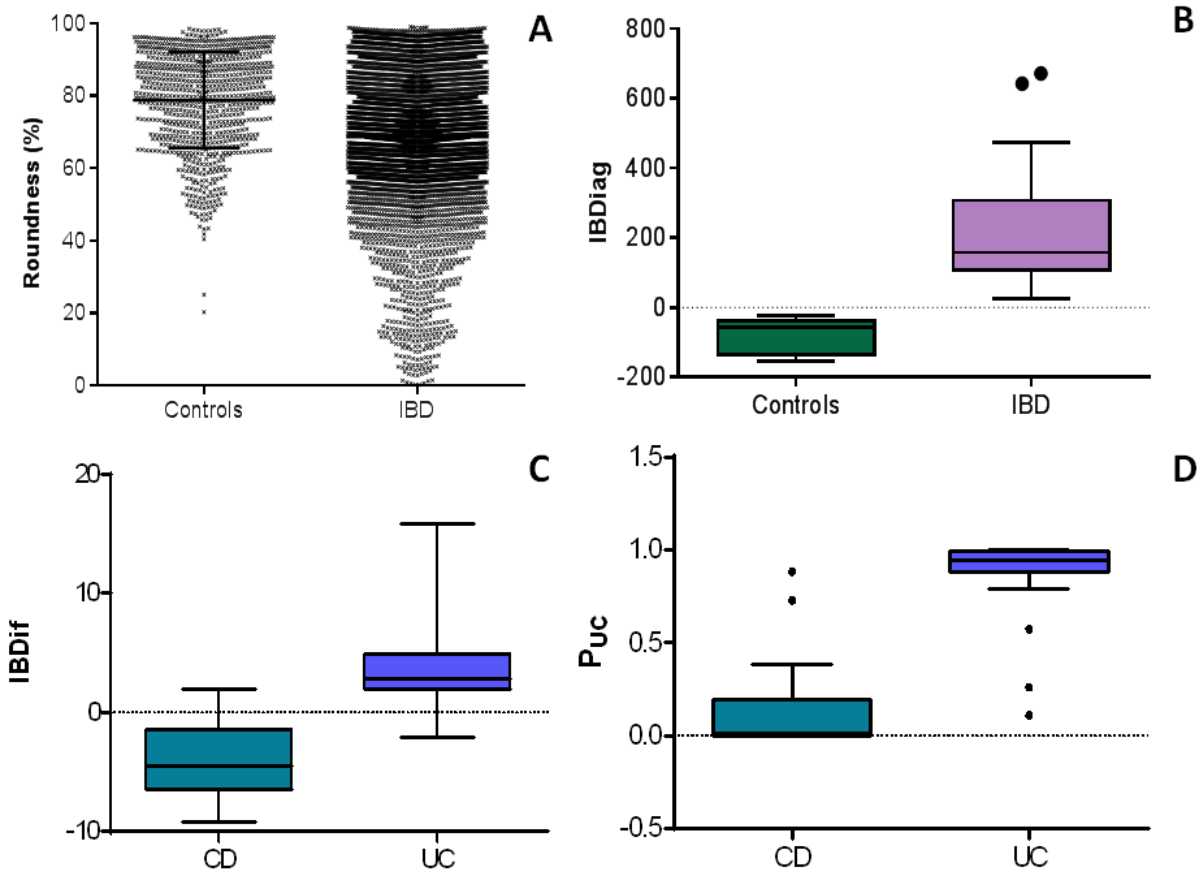


Figure 4: A) Repartition of the roundness values, comparing IBD and controls patients, notice the existence of greater variations of values within the IBD group. B) Results of the IBDiag score comparing controls and IBD patients. C) Results of the IBDif score, comparing CD and UC patients. D) Probability toproperly diagnose UC rather than CD with the IBDif score compared between UC and CD patients.

| Parameter | Univariate AUROC | Multivariate AUROC ($+COV_{Feret}$) |
|---|---|---|
| **Perimeter** | 71.33 | 98.00 |
| **Sphericity** | 72.44 | 97.78 |
| **Roundness** | 65.56 | 98.00 |
| **Feret** | 79.22 | 98.44 |
| **EF** | 74.44 | 98.00 |
| **Ma/ma** | 68.00 | 98.22 |
| **Density** | 70.44 | 98.22 |
| **MVL** | 59.18 | 97.96 |
| **MVA** | 65.65 | 97.73 |
| **MVD** | 67.35 | 98.41 |
| **AICD** | 96.89 | 100.00 |
| **MICD** | 56.00 | 98.22 |
| **WT** | 96.67 | 100.00 |
| **FLCM** | 86.89 | 99.11 |
| **$COV_{Perimeter}$** | 92.44 | 98.67 |
| **$COV_{Sphericity}$** | 75.78 | 98.67 |
| **$COV_{Roundness}$** | 89.56 | 98.44 |
| **$COV_{Feret}$** | **98.00** | |
| **$COV_{EF}$** | 88.00 | 98.22 |
| **$COV_{Ma/ma}$** | 81.11 | 98.00 |
| **$COV_{Density}$** | 74.89 | 98.00 |
| **$COV_{MVL}$** | 89.33 | 98.44 |
| **$COV_{MVA}$** | 84.67 | 98.44 |
| **$COV_{MVD}$** | 81.33 | 99.33 |
| **$COV_{AICD}$** | 96.89 | 99.33 |
| **$COV_{MICD}$** | 88.89 | 98.89 |
| **$COV_{WT}$** | 80.89 | 97.78 |

Table 3: Areas under the curve of receiver operating characteristicfor each parameter and COV for the positive diagnosis of IBD.

| Parameter | Univariate AUROC | Multivariate AUROC | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | + WT | + FLCM | + $COV_{WT}$ | + $COV_{MVA}$ | + $COV_{Perimeter}$ | + MICD | + MVL |
| **Perimeter** | 63.29 | 74.56 | 81.16 | 85.83 | 88.08 | 91.95 | 95.81 | 95.81 |
| **Sphericity** | 53.38 | 74.40 | 80.84 | 86.96 | 88.57 | 90.50 | 93.56 | 93.56 |
| **Roundness** | 59.98 | 74.24 | 81.00 | 85.51 | 87.44 | 91.95 | 94.69 | 94.69 |
| **Feret** | 63.77 | 73.91 | 81.48 | 85.99 | 88.08 | 92.59 | 95.65 | 95.65 |
| **EF** | 66.26 | 74.72 | 81.96 | 85.83 | 88.41 | 93.24 | 95.17 | 95.17 |
| **Ma/ma** | 65.30 | 73.11 | 81.16 | 85.67 | 87.76 | 92.59 | 94.36 | 94.36 |
| **Density** | 65.54 | 74.72 | 80.19 | 85.67 | 88.08 | 90.34 | 93.72 | 93.72 |
| **MVL** | 68.39 | 78.26 | 84.45 | 87.46 | 89.63 | 93.65 | **97.16** | |
| **MVA** | 64.97 | 76.92 | 82.27 | 87.46 | 89.80 | 92.64 | 96.49 | 96.49 |
| **MVD** | 60.20 | 74.58 | 80.60 | 84.62 | 87.12 | 90.64 | 93.48 | 93.48 |
| **AICD** | 69.08 | 76.01 | 80.52 | 86.31 | 88.24 | 90.98 | 94.36 | 94.36 |
| **MICD** | 71.98 | 73.11 | 82.61 | 87.76 | 88.89 | **93.72** | | |
| **WT** | 74.40 | | | | | | | |
| **FLCM** | 72.06 | **80.52** | | | | | | |
| $COV_{Perimeter}$ | 54.27 | 77.62 | 81.64 | 87.92 | **90.50** | | | |
| $COV_{Sphericity}$ | 57.49 | 75.04 | 80.19 | 85.51 | 87.76 | 93.24 | 95.49 | 95.49 |
| $COV_{Roundness}$ | 60.63 | 74.72 | 80.19 | 86.15 | 87.28 | 92.11 | 94.85 | 94.85 |
| $COV_{Feret}$ | 52.50 | 76.65 | 81.96 | 87.60 | 90.02 | 91.63 | 94.04 | 94.04 |
| $COV_{EF}$ | 60.63 | 73.43 | 81.00 | 85.83 | 87.76 | 92.11 | 94.20 | 94.20 |
| $COV_{Ma/ma}$ | 60.95 | 73.43 | 80.84 | 85.99 | 87.76 | 92.11 | 94.20 | 94.20 |
| $COV_{Density}$ | 73.35 | 77.46 | 82.93 | 88.41 | 89.86 | 93.56 | 95.97 | 95.97 |
| $COV_{MVL}$ | 52.74 | 75.36 | 80.52 | 88.24 | 88.24 | 90.02 | 93.56 | 93.56 |
| $COV_{MVA}$ | 49.44 | 75.04 | 80.84 | **88.57** | | | | |
| $COV_{MVD}$ | 60.87 | 79.23 | 83.74 | 87.92 | 89.53 | 93.40 | 95.81 | 95.81 |
| $COV_{AICD}$ | 55.23 | 75.20 | 85.19 | 86.15 | 88.08 | 90.50 | 93.72 | 93.72 |
| $COV_{MICD}$ | 54.43 | 73.43 | 80.35 | 87.28 | 88.89 | 91.30 | 95.17 | 95.17 |
| $COV_{WT}$ | 72.30 | 76.97 | **85.67** | | | | | |

Table 4:Multivariate areas under the curve of receiver operating characteristicfor each parameter and COV for the differential diagnosis of IBD.

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Vu, le Président du Jury,

(tampon et signature)

Vu, le Directeur de Thèse,

(tampon et signature)

Vu, le Doyen de la Faculté,

(tampon et signature)

# SERMENT MEDICAL

Au moment d'être admis à exercer la médecine, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité.

Mon premier souci sera de rétablir, de préserver ou de promouvoir la santé dans tous ses éléments, physiques et mentaux, individuels et sociaux.

Je respecterai toutes les personnes, leur autonomie et leur volonté, sans aucune discrimination selon leur état ou leurs convictions. J'interviendrai pour les protéger si elles sont affaiblies, vulnérables ou menacées dans leur intégrité ou leur dignité. Même sous la contrainte, je ne ferai pas usage de mes connaissances contre les lois de l'humanité.

J'informerai les patients des décisions envisagées, de leurs raisons et de leurs conséquences. Je ne tromperai jamais leur confiance et n'exploiterai pas le pouvoir hérité des circonstances pour forcer les consciences.

Je donnerai mes soins à l'indigent et à quiconque me les demandera. Je ne me laisserai pas influencer par la soif du gain ou la recherche de la gloire.

Admis dans l'intimité des personnes, je tairai les secrets qui me seront confiés. Reçu à l'intérieur des maisons, je respecterai les secrets des foyers et ma conduite ne servira pas à corrompre les mœurs.

Je ferai tout pour soulager les souffrances. Je ne prolongerai pas abusivement les agonies. Je ne provoquerai jamais la mort délibérément.

Je préserverai l'indépendance nécessaire à l'accomplissement de ma mission. Je n'entreprendrai rien qui dépasse mes compétences. Je les entretiendrai et les perfectionnerai pour assurer au mieux les services qui me seront demandés.

J'apporterai mon aide à mes confrères ainsi qu'à leurs familles dans l'adversité.

Que les hommes et mes confrères m'accordent leur estime si je suis fidèle à mes promesses ; que je sois déshonoré et méprisé si j'y manque.

NOM: DAVID PRENOM: Grégoire

# New endomicroscopic computer-based algorithm for the differential diagnosis of inflammatory bowel diseases.

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## ABSTRACT

**Introduction :** Unclassified inflammatory bowel disease (UIBD) remains a contreversial subject and up to 10% of IBD patients may still not receive an optimal therapy. In this contexte, endomicroscopy appears as a promising tool; but despite an increasing number indications, a great heterogeneity of use persists and it remains an expert technique.

**Objectives :** To define new computer-based quantitative and objective endomicroscopic bio-markers and assess the positive and differential diagnosis of IBD.

**Methods :** We analyzed a total of 409 movies recorded among 59 patients (9 controls, 23 Crohn's disease (CD) and 27 ulcerative colitis(UC)). The comparison between groups was done using thirteen architectural parameters: the perimeter, the sphericity, the roundness, the elongation factor, the maximal Feret diameter, the Ma/ma ratio, the density, the average and minimal inter-cryptic distance (AICD and MICD), the wall thickness, the mean area, length and diameter of the vessels and a functionnal parameter: the fluorescein leakage through the colonic mucosa (FLCM).

**Results :** Using the parameters described here and computer-based analysis, we developed two scoring systems : the IBDiag score that allowed the positive diagnosis of IBD among control patients in 100% of cases and the IBDif score for the differential diagnosis between UC and CD in remission with a sensitivity of 92.3% and a specificity of 91.3%.

**Conclusion :** Quantitative and objective parameters can diagnose IBD and differentiate UC from CD. This observation confirm the potential of endomicroscopy in this indication and the possibility to overcome the observer expertise by computerized measures.


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## KEYWORDS

Endomicroscopy - Inflammatory bowel disease - Unclassified colitis